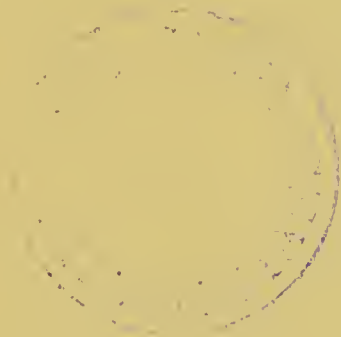


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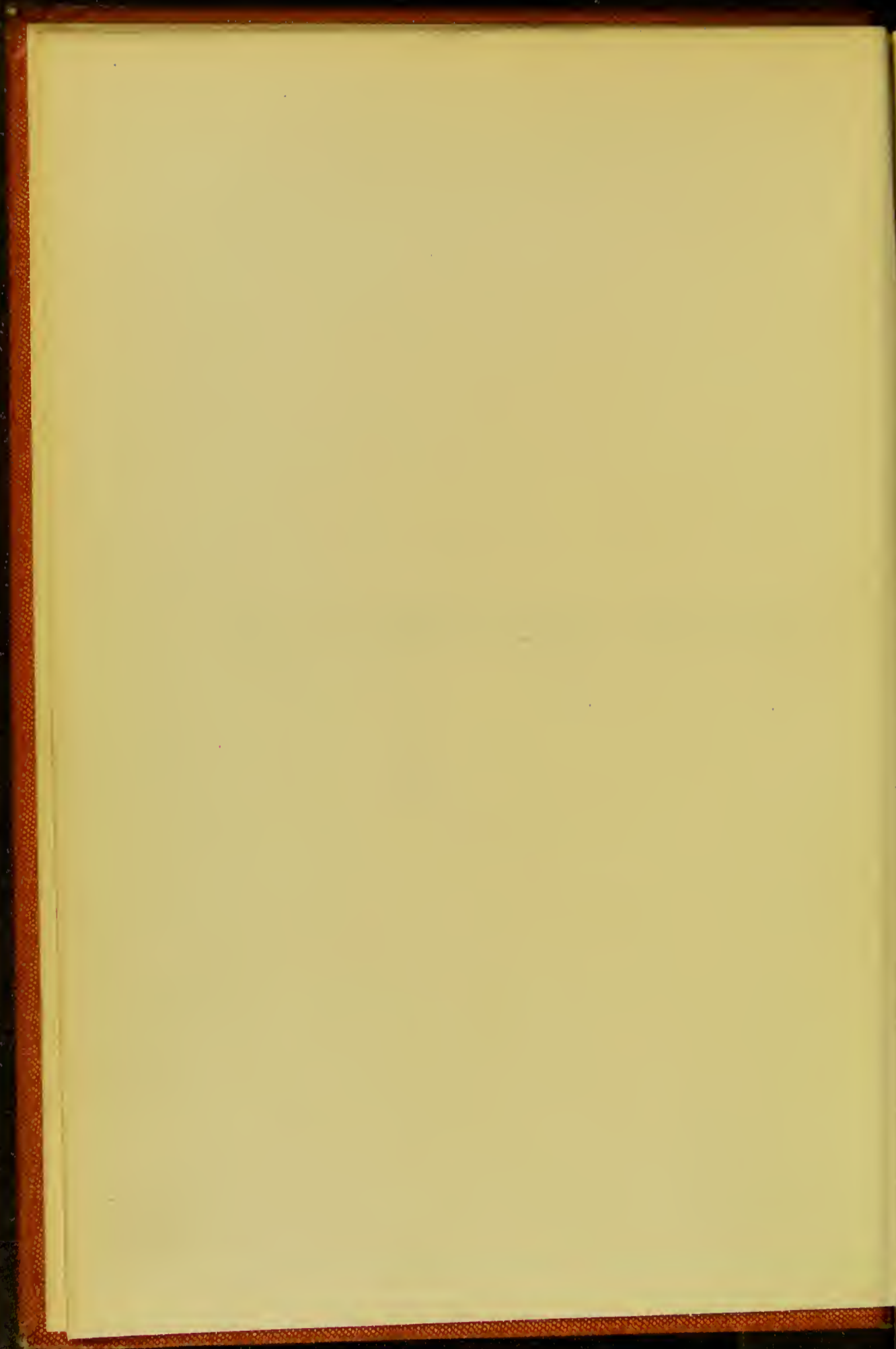
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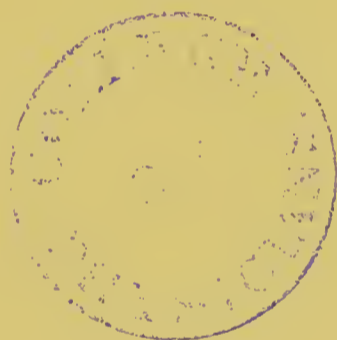
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THE CELL AS THE UNIT

THE CELL AS THE UNIT OF LIFE







[Photo. Elliott & Fry.]

ALLAN MACFADYEN.

Born May 26, 1860, in Glasgow. Died March 1, 1907, in London.

THE CELL AS THE UNIT OF LIFE

AND OTHER LECTURES

DELIVERED AT THE ROYAL INSTITUTION,
LONDON, 1899-1902

AN INTRODUCTION TO BIOLOGY

BY THE LATE

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PREFACE

The Courses of Lectures published here are some of those delivered by the late Dr. B. B. Barnard at the Royal Institution, London, during his tenure of the office of Fullerian Professor of Physiology. The time of delivery created considerable interest, and it has been thought that their issue in book form would be a fitting tribute to some memento of a life full of public service, which will not be all too soon.

I was requested to act as Editor, and I have consented, having been a friend of Dr. Barnard, and associated with him in his work for many years. The task has proved a somewhat difficult one, and I therefore ask the indulgence of the reader.

The lectures are reproduced nearly as they were delivered, for I have made no alterations either in the matter or in the style. Each course of lectures is complete, and I am obliged to the reader to note that there is necessarily some repetition.

I am indebted to Dr. B. B. Barnard for some details of Dr. Macfadyen's early career, and to Mr. Barnard for photographs from which the illustrations have been reproduced: and to Mr. the Editor of the 'Journal of Hygiene' for the block from which the portrait of Dr. Barnard (Messrs. Elliott & Fry) is reproduced.

King's College, London: November 1924.

PREFACE

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R. T. H.

King's College, London : September 1908.

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BIOGRAPHICAL NOTICE

Allan Macfadyen, the author of these Lectures, was born in Glasgow, and was educated in Edinburgh at Dr. Bryce's Collegiate School.

He entered the University of Edinburgh in 1878, and studied science and medicine under such well-known teachers as Professor Hutton Balfour and Dr. (now Professor) Isaac Bailey Balfour (Botany), Professor Sir Wyville Thomson and Professor Nicholson (Zoology), Professor Crum Brown (Chemistry), Professor Rutherford (Physiology), Professor Sir William Turner (Anatomy), Professor Hamilton (Pathology), Professor Sir T. Grainger Stewart (Medicine), Sir Alexander Simpson and Sir Croom Halliday (Midwifery), Professors Chiene and Annandale and Dr. Joseph Bell (Surgery), Dr. Clouston (Mental Diseases), Dr. Argyll Robertson (Ophthalmology), and Sir Douglas MacLagan and Sir Henry Littlejohn (Medical Jurisprudence). He also attended Professor Masson's course in English Literature, and to the last retained his love of good literature. Dr. Macfadyen did not often sit for the class examinations, which were then not compulsory, and so gained few class prizes.

Dr. Macfadyen obtained the M.B., C.M. degrees in 1883, and graduated as Doctor of Medicine in 1886,

gaining the gold medal, and subsequently obtained the Bachelor of Science degree in Hygiene. He then proceeded to the Continent, and studied at Berne, Göttingen, and Munich under such masters as Pettenkofer, Nencki, and Carl Flügge. He thus gained an intimate knowledge of chemical and bacteriological methods and imbibed that love of research which prompted his later investigations, as well as obtaining a mastery of the German language, which helped him not a little in the course of his subsequent work.

From 1889 to 1892 Dr. Macfadyen was a Research Scholar of the Grocers' Company, and during his tenure of the scholarship investigated the ferment action of bacteria and the chemical action of bacteria on albumens and peptones. About this time he became Lecturer on Bacteriology at the College of State Medicine, London, which had been established with the object of promoting the study of preventive medicine and the education of medical men and others in the principles of hygiene. The College of State Medicine was subsequently amalgamated with the British Institute of Preventive Medicine, of which it formed the nucleus, an Institute founded as a memorial of the work of Pasteur and modelled on the plan of the great Continental institutes of hygiene. Here he was the head of the Bacteriological Department and later became Secretary on the resignation of Dr. Ruffer. In the latter capacity Dr. Macfadyen had a large share in the planning and organisation of the Institute's fine new building on the Chelsea Embankment, now known as the Lister Institute of Preventive Medicine. At Chelsea he acted as Secretary to the Governing Body

of the Institute and was head of the Bacteriological Department, a post he held until 1906, when, under circumstances into which it is unnecessary to enter here, he resigned his position and devoted himself to original research, in the prosecution of which he fell a victim to his zeal, accidentally infecting himself with typhoid and Mediterranean fevers, of which he died on March 1, 1907, at the early age of forty-six, a martyr to that science he loved so well and to which he gave the best years of his life.

One of Dr. Macfadyen's earliest contributions to science was on the behaviour of bacteria in the digestive tract. In this he showed that the gastric juice and intestinal secretion possess little power to protect the body against invasion by micro-organisms which happen to find their way into the alimentary canal, that the intact wall of the intestine is usually an efficient barrier against the entrance of micro-organisms, and that the acids in the food play a part in preventing abnormal and undue fermentation in the digestive tract.¹ This was followed by a joint paper

¹ Macfadyen says in the paper referred to (*Journ. Anat. & Physiol.* xxi. p. 437): 'The researches show that the organic acids of the food have a not unimportant influence over bacteria. They probably hinder the development of fermentations by killing or paralysing the germs. It seems as if they could have the power to kill out extraneous bacteria which have nothing to do with the intestinal processes, and to leave the path free for the usual fermentations. This may also explain their importance as dietetics, *e.g.*, *sour milk*, vinegar, pickles, mustard. Clinically, too, in abnormal fermentations, *lactic* or acetic acids might be given instead of hydrochloric acid.' This quotation is of interest at the present time in view of the fact that Metchnikoff has recently advocated the use of buttermilk, or of milk curdled with a particular lactic acid-forming bacterium, as a protective against abnormal fermentations in the digestive tract, which he believes are largely responsible for the onset of senility.—ED.

with Professor Nencki and Dr. Sieber on the chemical processes occurring in the small intestine of man, and in particular the share taken by the numerous bacteria present in the decomposition of the food ingested. He also studied the action of bacteria on proteid bodies, and, with Sir Lauder Brunton, the ferment action of bacteria, showing that peptonising and diastatic enzymes are produced by many micro-organisms, and this work was continued in a paper on the biology of the ringworm organism, published in 1895. The thermophilic¹ and photogenic² bacteria also attracted Dr. Macfadyen's attention, and important contributions to the biology of these organisms were made in collaboration with Dr. Blaxall and Mr. J. E. Barnard respectively. The Pasteurisation of milk, the bacillus of bovine and of human tuberculosis, the phosphorescent bacteria, and problems of disinfection were some of the subjects to which from time to time he devoted his attention. Not only did he himself contribute much to science, but many of the researches which emanated from the Lister Institute while Dr. Macfadyen was there were due to his inspiration. Cellular problems, and, in particular, the cellular constituents of micro-organisms, always had a fascination for him; and Dr. Macfadyen's claim to a niche in the temple of fame in the department of bacteriology undoubtedly rests on his work on the intracellular juices, the 'endotoxins,' of certain disease-producing micro-organisms.³ His natural

¹ Bacteria thriving at the high temperature of 140° F. (See p. 365.)

² Bacteria which develop a phosphorescent light during their growth. (See p. 257.)

³ The poisons generated by disease-producing microbes are known as 'toxins.' Some of these toxins are found in the medium in which the

bent in this direction received an impetus from the researches of E. Buchner on the intracellular alcoholic ferment of yeast, the 'yeast zymase,'¹ published in 1897-1900; and, with Mr. Sydney Rowland, a machine was devised for grinding up the yeast cells, and Buchner's results were in the main confirmed in a paper published, in collaboration with the late Dr. Harris Morris and Mr. Rowland, in the 'Proceedings of the Royal Society, London.' The apparatus employed in this research for grinding up the yeast cells formed the nucleus of the machine which was ultimately evolved, with the aid of Mr. Rowland, for triturating and disintegrating the much smaller bacterial cells in the presence of liquid air, the latter being used to freeze the cellular constituents hard and render them brittle, as well as to inhibit chemical change in the liberated juice.

The introduction of the antitoxic treatment of disease in 1894, and its great success in the treatment of diphtheria,² suggested that for every infective disease the specific micro-organism of which was known, it would be possible to prepare an antitoxin or curative serum. This, however, unfortunately proved to be incorrect, and by the year 1900 or thereabouts it was recognised that unless some new principle were evolved no advance could be hoped for in the serum treatment of disease. Dr. Macfadyen conceived that if the bacterial cells were triturated and ground up, and their intracellular constituents, or organism is growing, as in the case of diphtheria and tetanus; but in many instances no toxin is excreted, as in typhoid and cholera, the toxin apparently being intimately associated with the protoplasm of the bacterial cells. Such intracellular toxins are known as endotoxins.

¹ See p. 225.

² See p. 334.

‘endotoxins,’ as they may be termed in the case of many disease-producing micro-organisms, thus liberated, the injection of the endotoxin into an animal might be followed by the formation of an ‘anti-endotoxin,’ analogous to diphtheria antitoxin, which is formed when the diphtheria toxin is injected into a horse,¹ and that this anti-endotoxic serum could be used in the treatment of such diseases as typhoid fever, pneumonia, blood-poisoning, &c. With the aid of his colleagues, Mr. Rowland and Mr. Barnard, and of his laboratory assistants, Messrs. Burgess and Thompson, apparatus and methods were gradually evolved which enabled Dr. Macfadyen, with the machine mentioned above, to obtain the bacterial juices or endotoxins apart from the bacterial cells, and without the use of heat and chemical agents and extractives, which alter more or less their characters, chemical changes in the bacterial juices being inhibited by the intense cold of the liquid air employed in the process.²

In a series of papers which appeared during the last four or five years of his life, Dr. Macfadyen showed that the intracellular juices of virulent disease-producing microbes are intensely toxic, and may be regarded as the true toxins (endotoxins) of the organisms; that an animal injected with sub-lethal doses of an endotoxin becomes immunised or insusceptible to the endotoxin, and also to the microbe from which the endotoxin is derived; and that the serum of such a treated animal has antitoxic properties, conferring immunity and curing infection

¹ See p. 331.

² Sir James Dewar has shown that chemical changes are almost or quite inhibited at this temperature (300° F. below zero).

with the organism. It was just when his work had reached this important stage that Dr. Macfadyen contracted his fatal illness, so that the ultimate value of his method must, alas! be left to others to work out. During this period in which he was engaged on these important researches, Dr. Macfadyen's attention was naturally directed to the effects of low temperatures on bacterial life. With the help of Sir James Dewar, various bacteria were subjected to the intense cold of liquid air for long periods, and later to that of liquid hydrogen, and the astonishing result was arrived at that these extreme temperatures have little or no effect on the vitality and functions of the organisms submitted to their action.¹

It was in 1901 that Dr. Macfadyen was elected to the Fullerian Professorship of Physiology in the Royal Institution, London, and the lectures included in this volume are some of the courses which were delivered during his tenure of this post. Throughout them all the paramount importance of the *cell* is the dominant idea, the belief is again and again reiterated that the fundamental problems of biology are centred in the cell, and that every physiological and every pathological problem is ultimately a cellular one.—R. T. H.

¹ See p. 371.

THE CELL AS THE UNIT OF LIFE

A COURSE OF FIVE LECTURES

DELIVERED AT THE ROYAL INSTITUTION

FEBRUARY AND MARCH 1901

LECTURE I.

Introduction—All Vital Phenomena centred in the Cell—Characteristics of Living Matter—Growth of Physiological Knowledge—Vital Force—Conservation of Matter and of Energy—Cellular Physiology—Protoplasm—Cell-structure.

I should like to express the pleasure I feel in engaging for the first time on the duties of the Fullerian Professorship of Physiology at the Royal Institution. The position brings with it not only a feeling of pleasure, but likewise, as I am fully conscious, a feeling of responsibility. The pleasure will consist in laying before you from time to time the results of various branches of inquiry, in so far as these have a special bearing on the one subject that is of perennial interest to us all—Life and its phenomena. The study of Life is beset with many peculiar difficulties as regards the investigation of its expressions and the explanation of their origin. It is not my intention to minimise these, or, in the face of so many abstruse problems, to set up a series of generalisations which might be of a more or less misleading character. There is nothing to which we give our serious attention in the realm of Nature that is easy to understand, nor can it be hoped to develop in such branches of inquiry what might be called ‘Science without Tears.’ The endeavour is to get behind the effect to the cause with the aid of the

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senses we possess, and we must use them conscious not only of their opportunities but likewise of their limitations. We are called upon to make the best use of our wits, and it is only by clear thought that we can hope to attain to clear exposition. And in this respect it is a great advantage to the specialist to be able from time to time to address a more general audience, and to rid himself thereby of the purely pedantic—that laboratory mildew which is apt at times to settle on the best of us—and which usually consists in endeavouring to make the part appear to be greater than the whole. At the same time certain of the great impulses of the Victorian Era have been as a breath of fresh wind—the Darwinian Theory, the Atomic Theory, and the Doctrine of the Conservation of Energy and of Matter. They have not only stimulated minute and special research, they have broadened our conceptions and point of view. Whatever the subject, scientific inquiry now runs in under channels more than it has ever done before, and the mental attitude of the darker ages which lasted so very long has become unintelligible. To this must be added the modern spirit of research which only finds satisfaction in knowledge that rests on an accurate *experimental* basis.

The responsibility to which I have referred has been laid upon me by my predecessors in the present office, and it constitutes a stimulus to do the best that lies in one's power. I have considered the lines that it would be best to follow, and I may be allowed at this moment to give a brief outline of the direction these will take. I have resolved to take Physiology in the widest sense of the word, and not to confine myself to any one special branch of

its study. We have a Vegetable Physiology, an Animal Physiology, and, most important of all, a Human Physiology, and their several problems meet us wherever there is Life and its Functions to be studied and described. Each of these branches has become a large and weighty discipline charged with many facts of interest and importance. Further, each branch has become subdivided into lines of research which individually would furnish more than ample material for many courses of lectures. I might dilate on Human Physiology—the phenomena of muscle, brain, nerve tissue, &c.—and confine my observations to one or other of these functional structures. If, however, the phenomena of Life be regarded as a whole, one finds that there are not only in its physical basis but likewise in its functional activities relationships and resemblances. There is no vital phenomenon as seen in its most complex and highest manifestations which has not its counterpart, nay its origin, in some more elementary or humbler living object. There is a solidarity in the essential phenomena of Life which is shared not only by the simple *Amœba*, but by the most highly specialised cell in a tissue of the human body. I am convinced that we could not find a more appropriate subject for discussion in this and in subsequent courses of lectures than to devote our attention to the connecting threads that run through the web, and to consider the phenomena of Life from the broad point of view of General Physiology. And in consulting the existing literature I have been struck by the great accumulation of facts that has been brought about by individual workers in recent years. It would be both useful and instructive to correlate these as regards the

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main issues in question, so far as it is in one's power to do so. I feel sure that those of us who are engaged in specialised lines of investigation will agree that such attempts from time to time are of the greatest value. No one can regard the general results in any branch of inquiry without finding therein something which throws a valuable sidelight on his own special investigations or which furnishes a stimulating thought. The attempt could not be more appropriately made than in this Institution. And I think we shall find ourselves continuously touching upon points which all physiological investigations have in common, however specialised the lines of inquiry may be. There are already facts enough to justify the experiment upon which I propose to embark in attempting to trace the Physiology of a Common Life. I am conscious of others who have synthesised so admirably the knowledge of their time, notably Claude Bernard in 'Les phénomènes de la Vie,' and recently Professor Verworn in his text-book of 'Allgemeine Physiologie.' To these writers and to many others I am indebted for numerous valuable data which necessarily must lie outside the narrow sphere of one's own personal observation.

All vital phenomena have their bases in the *Cell*, whether it exists as a free individual, as a colony, or as a republic of cells in the tissues and organs of the human body. Every inquiry as to Vital Function, however complex its nature, leads us back to the cell as the primary basis of Living Energy. The present course of lectures will therefore deal with the Cell as the Unit of Life. The endeavour will be made to trace the general phenomena of Life as manifested in the individual Cell—

touching upon the main phenomena and the general conceptions they afford us. In this way I hope we will be put in possession of a number of general facts which can be brought to bear on any individual manifestations of Life, whether these are to be found in the Plant or in the Animal. During the remainder of my tenure of this appointment I shall, whilst adhering to a general view of Vital processes, take up, and I hope be able to deal with, individual phases of the same and discuss them in more detail than is possible in the present course of lectures. The present course will, therefore, serve as a general introduction to the consideration of special questions—such, for example, as the production by the Cell of Ferments, Enzymes, Toxins, &c., and the bearing of these upon individual and extraneous Life. We will now pass from the prefatory remarks to the consideration of our present theme—the Cell as the Unit of Life. The title in itself suggests certain important conceptions, which are the fruit of long inquiry, and it will be necessary in the first instance to trace broadly their inception and the steps that have led to their present development. This will help us to understand the objects of Physiology, and the lines of Method and Inquiry these have given rise to. Physiology deals with the investigation of the phenomena of Life. We are all acquainted with the difference between a Rock and a Tree. If we were to put our notion into its elementary form we would probably say that the Tree grows and the Rock does not. This is what is apparent: We plant the stone and the seed in the ground; the one remains inert, the other exhibits an activity which is expressed in growth. We have here an

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elementary notion of one of the distinguishing features between a living and a lifeless object, which must have appealed to the senses of primitive Man. If we think further we note that a crystal likewise increases in size; there is an increase in bulk—a form of growth. To establish a difference, therefore, between the living and the lifeless object, growth in the gross interpretation of the word is too narrow a conception. If we further compare a bird and a stone we find that the former is gifted with motion, by means of which it is able to effect a change of position; the stone lies motionless in our hand, the bird escapes. In fact, our elementary conception of Life is intimately connected with the phenomenon of Motion. It is in some form of Motion that the phenomena of Life are transmitted to our senses. The wind, it is true, moves and the sea moves in virtue of the play of forces which are external to the air and to the water, but the supreme difference is that in the living object the guiding impulse comes from within, and expresses itself in some form of *purposeful activity*. When the motion of the bird permanently ceases we say it is dead. The loss of appreciable motion leads us to distinguish between what once was, and what is no longer, alive. We cannot apply to the apparently inert seed the tests of simple Growth and Motion. The earliest observation must, however, have taught that the planted seed grain possessed a quality that was not to be found in the sand grain—a property not merely of growth, but of development into a form representing the plant from which it originated, a replica of the parent in the daughter plant; an act of reproduction has been

performed. The seed had not simply been the play of general external forces; it had brought peculiar forces of its own into play. And in this we come to the bed-rock, to the fundamental difference between living and lifeless matter, and one which we are not able to reproduce with any of the chemical and physical means at our command. In the midst of a mechanical environment we find a spontaneous and purposeful activity, characterised not merely by Growth and Motion, but by the power of self-multiplication and reproduction and the transmission of the specific energy which we term Life. Our experience does not reach further than this: that Life produces Life, or, to put it in more definite language, that the existing cell postulates all preceding and all succeeding cells—*omnis cellula e cellulâ*. It is the one sure test of Life. And this is of great importance, as it enables us to get away from the appearance and nearer to the reality, from what grossly appeals to our senses (such as motion, growth, &c.) to the finer, subtler distinction that is based on a profoundly essential property. We get behind the phenomenon and appreciate, if we are unable to explain, the Cause, the nature of which Cause it has been sought to express in a phrase which is of purely abstract significance—*Vital Force*—a force which lies in the seed cell and not in the sand grain; an active, a generating, a reproductive power giving rise to something equally endowed with the same properties, more marked in the seedling, more subtle, but still there in the fresh seed. The anthropomorphic conceptions of earlier times with regard to Nature were based not so much on this subtle difference as on the coarser attributes of Life, such as Motion, and

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the animate conception that was thereby applied to external forces, such as Wind, Water, and Fire. I should like to repeat that in getting down to certain primary conceptions as to the attributes of living things, we have not by any means solved the problem. We have simply endeavoured to seize upon the cardinal property which distinguishes animate from inanimate objects, and which is perceptible to us by reason of its manifestations. The cause itself remains a phenomenon to be explained. When we come to deal with Human Physiology, we are brought face to face with the psychical phenomena and the Dualistic conception of Body and Mind, with which it is not our province to deal. Our point of view for the object we have in contemplation must be a Monistic one—viz., the essential unity of the particular phenomena we are investigating, under whatever varied disguises they may present themselves to us. Amongst the earliest doctrines concerning Life we find a *πνεῦμα*, or aërial substance, postulated, a fine material agent attracted by the lungs, and passing from them into the body. Galen expanded this doctrine. The phenomena of Life were due to three different forms of *πνεῦμα*, situated—(1) In the Brain and Nerves; (2) in the Heart; and (3) in the Liver. These were regenerated by the *πνεῦμα* of the air through the Lungs. In this we note an appreciation of the respiratory processes, and now, of course, we would put oxygen in the place of the hypothetical *πνεῦμα*. Galen likewise made a study of the normal structures on which functions are based. These attempts were mainly made with a view of aiding treatment, and for thirteen centuries Galen's System remained the great Codex of Medicine. It

was not till the sixteenth century that any real progress in ideas was made, when we note the work of Vesalius and others regarding the anatomical structure of the heart and the vessels. Then there came the outstanding discovery by Harvey of the Circulation of the Blood. The arteries and veins are connected by a system of capillary vessels, and the blood passes from the arteries to the veins by this capillary system and thence to the heart. The blood thus passes through the body in a closed circle. And with Harvey experiment acquired its proper place in Physiology. We have likewise Harvey's doctrine *de generatione animalium*, and the formulation of the great principle *omne vivum ex ovo*. In the seventeenth century a certain progress was made as regards an understanding of the phenomena of Life, *e.g.* as regards the nature of the processes of digestion; whilst in the seventeenth and eighteenth centuries there came a development of more exact methods of physiological research. Amongst these we may particularly note the discovery of the Microscope, and the observations made by Leeuwenhoek and others, in which lay the germs of the present great development in our knowledge of bacteria and other minute forms of Life. The name of Haller is a conspicuous one in the eighteenth century, and he embodied a number of facts and theories in his 'Elements of the Physiology of the Human Body.' Haller, in his Pre-formation Theory, put forward the view that the egg, for example, produces the complete animal, which exists therein pre-formed. This view was opposed by Wolff, who held that all organs are formed one after the other in the course of development, and do not pre-exist as such in the egg. And this, of

course, is the modern point of view. In the eighteenth century Brown advanced the Doctrine of the Excitability or Irritability of all living matter as a fundamental property distinguishing living from lifeless matter. This phenomenon led to the hypothesis of a special quality which resided in living matter and which had its special seat there—to wit, a Special Vital Force, a something beyond and above the mechanical properties of inanimate matter. This Doctrine of Vitalism has been, and still is, one that receives strong support. Though many doors have been forced, and as we penetrate deeper into the phenomena of Life much has become clear, we come ultimately to the seat of the phenomena—the individual cell—in which the phenomena cease to be analysable—a shut door, on the other side of which is the mystery of Life itself. This still remains for us a book with seven seals, which we may, or perhaps never may, be able to open and read. It is the most daring question ever formed by Conscious Life, What am I and whence am I? It was, however, by adhering to chemical and physical theories that the inquiring mind was able to elucidate a number of phenomena and to narrow down the tract of the unexplained. Discovery in the domains of physics and chemistry came to the aid of physiological inquiry. We have, for example, Galvani's discovery that Electricity is educed from the nerves. Likewise the discovery of oxygen and the fact of the assimilation of carbonic acid by plants, and the comparison made between respiration and the chemical process of combustion. And we have the most important discovery that certain nerves in the body control motor and sensory phenomena. Further, the emergence, by the

aid of microscopical and other methods, of the doctrine that Life does not arise spontaneously, but out of previously existing forms of life. At the commencement of the nineteenth century we have the classical name of Johannes Müller, in whose physiological writings may be found so many of the germs of present-day physiological inquiry. Whatever the essential nature of Vital Force might be, it followed in its manifestations Chemical and Physical Laws. Müller therefore endeavoured to explain the phenomena on mechanical principles. As has been said of him, 'He had a philosophical method of research, always keeping the general problems in view and regarding the special methods and questions solely as a means to one end—the attaining of a harmonic view of Nature.' We may especially note his doctrine of the specific energy of the organs of sense. The different stimuli, whatever their character, when exerted on the same organ—for instance, the eye—can only produce one and the same kind of impression, viz., that which is produced by the action on the organ in question of its natural stimulus, in this case Light. And, on the other hand, one and the same stimulus applied to various organs of sense produces different sensations according to the organs acted upon. The fundamental principle was demonstrated that the external only exists as it is perceptible through our organs of sense. The great developments in research and in experimental methods of inquiry rendered it impossible for research to maintain a central point of view or to develop harmoniously in the form of a Comparative Physiology. Physiology, including the highest of all, Human Physiology, was impelled to take directions, not

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only as regards the functions to be studied, but likewise as regards the methods to be employed. It was natural that these should follow two broad roads of inquiry—the one Physical and the other Chemical. We have, therefore, a development of Physiological Chemistry and Physiological Physics. Ludwig was one of the eminent creators of Physical Methods, such as the Graphic Method of investigating functions in their physical manifestations; and in France Marey did like service. Du Bois-Reymond developed the technique of galvanic stimulation in reference to the investigation of the physics of muscle and nerve. The experimental study of Life on the living object itself gave remarkable results in the hands of Claude Bernard and others. Bernard contributed much to the solution of general vital problems. Many other names might be recalled, but it would serve no useful purpose in our present considerations. One must, however, mention the ‘Cellular Pathology’ of Virchow, which, since its publication in 1858, has profoundly influenced the study of Physiology as well as of Pathology. The progress of Anatomy from the naked eye to the microscopical examination of the tissues and their elements has resulted in the development of Histology. This likewise became a natural trend in Zoology, in which the microscope is an essential agent in research. And we must not omit to mention one of the most important and significant lines of research, which no student of physiology can afford to neglect, viz., the study of cellular development and organisation, or, as it has been termed, Embryology. Here, likewise, the microscope is an indispensable adjunct. In the main, pure Animal Physiology has kept to the study of certain living test

objects. In Botany the study of plant function has undergone a remarkable development, and to this study many are devoting their whole energy. A knowledge of the essential features of Plant Physiology ought to be acquired by anyone desiring to understand fully the elementary phenomena of Life, for the conditions in the plant are less complicated and permit of readier investigation, and the methods which can be applied are also of a very varied character. In no domain can cellular physiology be more profitably studied or with greater reward as regards general problems. The microscope is also in this department a *sine qua non*.

The Doctrines of the Conservation of *Matter* and of *Energy* have influenced the physiological as well as other fields of research. Though the form it assumes may vary, matter is not destroyed, its amount remains constant. As in the case of water, we may have it existing in a solid, a fluid, or a gaseous form. If it disappears as such, it is simply due to a dissociation of the material elements, Hydrogen and Oxygen, of which the molecule of water is composed. They still exist, and in the same amount, to re-combine as water or to enter into the constitution of other kinds of matter. This applies equally to the elements of which a living tissue is composed. Similarly Energy, like Matter, cannot be destroyed; it remains constant—there is a conservation of Energy. It simply undergoes different phases, such as Light, Heat, &c., and in its main conditions it may be Actual and Kinetic, or be Potential, Energy. This law likewise applies to living things, so far as tested. There is an equivalence between the energy derived from the food

and the heat developed by the body. To bind up these views of Force and Matter, we have the Atomic Theory. The atom is the smallest indivisible portion of matter, and the aggregate condition of Atoms finds its expression in the word Molecule. We have therefore, according to physical and chemical hypothesis, to regard material phenomena as the result of atomic movements and to consider matter as the seat of Atomic and Molecular forces, which with adequate knowledge can be expressed in terms of Mathematics. The atom is the seat of the play of Force or Energy. Every material phenomenon is the result of another material phenomenon. This enables us to find and to postulate definite laws which matter will follow under definite conditions, and by the aid of our sense perceptions to elaborate a doctrine of *Causality*. In the animate world the great doctrine has been established that organisms are built up of Cells, and that each and every organism develops from a *Cell*. The cell, therefore, is the centre and the origin of Life processes. We have finally the Law of Descent in the Animal World. The various forms of organic Life are not isolated units, they are related to one another. The complex have had their origin in simpler forms, and life in its present versatile aspects is the result of a long process of evolution. This has revolutionised the Study of Structure, and as regards function we see its influence on the consideration of problems of heredity. These are the great ideas which have mainly moulded modern scientific thought into the channels in which it now runs.

In Physiological inquiry the object is to find a reasoned explanation of the phenomena of Life—to trace

them to their elementary causes or to trace the causal relationships and the laws that govern them. If we do not thereby come to an understanding of the ultimate cause, we are able to correlate and to understand its expressions. The methods employed will depend on the object in question and the aim in view. The important point is to select the right method for the special inquiry. In this respect, though much has been achieved, a great deal still remains to be done in the elaboration and application of physical and chemical methods to the study of Life. In this respect the resources of one science may help in the elucidation of another. The finer chemical decompositions may be accomplished by purely bacteriological agencies in a way that is impossible to the ordinary methods of chemistry. And, on the other hand, the methods of pure chemistry may reveal secrets of chemical metabolism in the living body. How much light has been thrown on the processes of the body by the decomposition and synthesis of Urea, and upon the complicated structure of the proteid molecule by the splitting and analytical dissecting power of microbes ! And as regards the study of the living substance itself, how much can undoubtedly yet be done by an elaboration of purely mechanical methods of cell disintegration, which will not expose it to the same degree to the modifying influence of chemical and other processes ! But there always comes a time when the possibilities of a given method seem to be exhausted. This is a dangerous period, and may induce not progress, but stagnation. The man, therefore, who is able to introduce a new method deserves well of his scientific brethren. The reason why we cannot open

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some doors is because the key will not fit the lock. See, for example, what the methods, elaborated notably at this Institute, have done for the study of the physics of gases, and Koch's methods of solid culture media for the study of bacteria, as well as the Graphic Methods for Physiological Inquiry. At the same time, no method is final, and it may be imperfect or useless for the object in view. There is therefore no one method in scientific inquiry. The man who can devise and modify his methods for the special object in view is the one who will push his knowledge furthest, in this way endeavouring to cultivate an elasticity, not a rigidity, in his mental attitude and in his methods of work. This valuable quality distinguishes the path-finders from the path-followers—it is the distinction between the finder of the precious stone and the one who polishes the facets. The methods of Physiological Inquiry have been in great part devoted to the study of the effect of stimuli on the movements of the body tissues—*e.g.* the galvanic current in its effect on muscle and nerve, along with the graphic methods of recording effects. There has been an application of Physics in elucidating the physical accomplishments of the body, as well as the results of Vivisection in explaining function. In Physiological Chemistry, the chemical methods have been directed to the study of the composition of living substance and the nature of its products. This branch has made great advances in the study not only of healthy, but of morbid, life. The life processes of plants furnish Physiological Chemistry with the richest material. The results of the past have led to a conception of the main manifestations of function in the body.

The significance of the respiratory processes are understood, the function of the lungs as bellows, with the resultant transference of oxygen to the body tissues by means of the blood cells. We further know that respiration, whether with or without a complicated apparatus, is a general phenomenon of Life, that every organism and every cell requires oxygen for its life processes, and that this craving is satisfied in one way or another. Respiration is an essential and a common feature of Life. The circulation of the blood is known, and the means whereby it is brought about. The circulation of food, &c., is found both in low and high forms of Life in the animal and vegetable world. Digestion has been studied as a chemical process, as well as the secretory products of the cells, which convert the food by chemical changes into an assimilable form. This chemical process occurs in the animal and the plant, as well as in the humblest cell. It is a universal phenomenon of Life, yielding absorbable substances, and conveying indispensable energy to the machine. The soluble bodies producing these changes can be separated from the cell, and their action demonstrated in the test-tube. Such soluble products are termed Enzymes. Their study is one of the most alluring character, on account of their derivation from and close relation to the living protoplasm. The passage of the assimilated food to the various tissue cells and the means whereby this is carried out are known to us. The mechanical principles governing body movements have been studied, and a proof furnished of the conservation of energy in the living body. The special senses with the physical laws that govern them have been investigated, and in the higher

nerve centres the localisation of function has been carried far. All these results are contained in the present Codex of Physiology. It is not our purpose here to dwell upon these results as regards the highly organised animals, or to deal with the special physiology of Man. All these results have brought us face to face with a notable fact which is dominating present-day inquiry. What is and where is the seat of all these diverse phenomena? The muscle moves, and locomotion is due to muscular movement. And why does the muscle move? It is expressed in a phase of contraction and expansion of its tissue—a property of the living substance which rests in the cells of which the tissue is composed. In the same way, digestion is a cellular act, and its specific products arise from the Cell. And the choice or rejection of given elements of nutriment is a *selective* act on the part of the living protoplasm of the cell. Similarly, with the organs of sense we trace the phenomena back to the nerve and ganglion cells. It is the Cell to which all these different paths lead us with the problem still to solve; but at the same time with the conviction that the phenomena have there their prime origin, and that we have reached the Unit of Life. As Bunge says, ‘All the processes in our organism which can be explained on mechanical principles are as little phenomena of Life as the movements of leaves and branches on a tree when shaken by a storm.’ It is when we can get so far as this that we begin to realise the limits of our methods, and consequently of our knowledge. There are forces which we have not been able to explain by Physical and Chemical Laws, and most notably the Springs of Vital Activity as they exist in the

cell. The phenomena of absorption would, no doubt, meet with a very good explanation on the principle of diffusion and osmosis through a membrane. But, on examination, it is found that the intestinal wall does not behave like a parchment membrane in the process—the protoplasm of the cell exhibits contraction and is actively engaged in the process—just as in the case of the simple *Amœba*. And we have already indicated the selective power of cells, as, for instance, in the case of the absorption into their substance of fat globules. These instances will suffice to show the value of the study of the cell in order to gain a just apprehension of the functions of Life. In the Cell the life processes have their seat; it is the Unit of Life. The appreciation of this has been fruitful in Botany and Physiological Botany, in Anatomy, in Zoology, and in Embryology. The study of unicellular plants and animals has given most valuable data. In the study of various functions the path leads from and to the Cell. That is, broadly speaking, a cardinal result in every branch of Vegetable and Animal Physiology. As one writer puts it, ‘In the muscle cell lies the riddle of heart movement and of muscular contraction; in the gland cell that of secretion; in the epithelial and in the white blood cell that of food absorption and assimilation; and in the ganglion cell the riddle of the regulation of Body phenomena.’ How much has been learned about important processes in the body by the study of the free wandering cells, the leucocytes, and amongst other things their hostile attitude towards bacteria, which they absorb and digest—a process which may be studied not only in the human body but in

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the frog's, and which is likewise evident in the simple *Amœba*!

It must be our constant endeavour to understand, and, if possible, to explain, the general and elementary phenomena on which all life processes are based. And I must say that I am in accord with those who fail to see how we are to reach this knowledge save through the attempt to found a Cellular Physiology, or rather a Comparative Cellular Physiology. This seems to me to be the great future problem, and to which the best energies may fitly be devoted in the future. The usual methods of preparing dead tissues, such as hardening and staining, will not lead us to the goal. The methods which would imply a study of the individual cells in the higher organisms involve their detachment and their observation under abnormal conditions. But, at any rate, I feel convinced that some of our most successful methods in the future will lie between the two extremes of Life and Death—*i.e.* by getting away from the dead fixed cell and pushing our methods and powers of observation several stages nearer to Life; in other words, by getting as close to Life as is possible. Such methods must in the first instance be more of a mechanical than a chemical nature. We have a recent example of what can be done in this direction in the methods employed for the rapid disintegration of Yeasts and in obtaining the fresh plasma of the Yeast cell. By this or by kindred methods many of the organs and tissues of the body might be studied, and perhaps a flood of light thrown on the metabolism of the cell. In the meanwhile the simple organisms, whether of plant or animal origin, always

present valuable material for study, and furnish us with a picture of the elementary phenomena of Life, as shared by every active cell. The essential phenomena are those of *Nutrition, Growth, Reproduction, Motion, and Sensation.*

The study of structure must precede that of function. We must have a knowledge of the substratum, of the physical basis of Life. And, as will be seen in subsequent lectures, the peculiarities in structure have a close relation to the vital processes of the cell. The methods of observation to be followed will, of course, be varied according to the object in view. The processes of cell division and multiplication will be observed with the aid of the microscope. By means of the hot stage the movements of single cells can be observed under the most favourable conditions, whilst the effects of gases and chemical and mechanical stimuli can in a similar fashion be noted. Even small vivisection experiments can be conducted on the cell, whilst the nature of intracellular bodies may frequently be determined with the aid of micro-chemical reagents. In this way a number of useful data may be obtained, not merely of special but of general import, with respect to vital processes—facts which help us to appreciate the solidarity of vital phenomena. The same processes we note in the simple cell are being conducted in the cells of the human body—respiration, metabolism, reproduction, &c. In the one instance the apparatus is simple, in the other it is complicated, but the processes are of a kindred nature, with the exception that each cell bears on it the impress of acquired and hereditary properties.

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It will be now fitting to turn for a moment to a consideration of the steps that have led to the development of the Cellular doctrine, and which have brought it to the pre-eminent position it holds at the present time. It is the foundation-stone of modern biology, and the centre of biological inquiry. We have termed the Cell the Unit of Life because it is the smallest known particle of living matter which can exhibit the essential phenomena of Life. The meaning which one attaches to the word *Cell* has undergone important changes, especially in its functional significance, since the time that it was first applied to the structural elements observed in animal and plant tissues.

It was by the study of the anatomy of plants that the discovery was made of the finer structure of tissues. Towards the end of the seventeenth century Malpighi, with the aid of magnifying glasses, noted that there were to be found in plants small chambers with solid walls, and containing a fluid—the *cellula*—as well as long vessels which permeated the substance of the plant. At the end of the eighteenth century Wolff and other observers discussed the question of the origin of plants, and came to the conclusion that the vessels were derived from the cells. In 1808 Treviranus proved that as regards the young parts of plants the vessels proceed from the cells. The young cells arrange themselves in rows, and form the tubes by the disappearance of the intervening cell walls. Further, the study of humbler plant life showed that Algæ could exist as single cells or as a small congeries of single cells. And at the beginning of the nineteenth century the cell had come to be recognised as the elementary

structural basis of the plant. In a botanical work of that period the following passage occurs:—‘The plant cells occur either singly, so that each constitutes a separate individual as in the case of the Algæ and Fungi, or they are united in more or less large masses so as to form the more highly organised plant. In this way each cell forms for itself an independent separate whole. It nourishes itself, it forms itself, and moulds the raw nutrient material taken up by it to very differing substances and shapes.’ In 1838 Schleiden sought to furnish the proof of how a cell originates, based on the discovery in 1833 by Brown of the cell-nucleus in certain cells of vegetable origin. Schleiden found the nucleus to exist in the cells of many plants, and to be an especially constant feature in young cells. He, therefore, regarded the nucleus as an important factor in the life history of the cell; whilst several observers suggested the application of the cell theory to animal tissues. This generalisation was first made by Schwann, who carried out and published in 1839 a series of microscopical investigations upon the agreement in structure and growth of plants and animals. Schwann laid it down as a principle that in the tissues the nucleus is the most characteristic and the least changeable constituent of the cell. He likewise studied the development of the tissues and the changes which the cell undergoes in the course of development into the adult animal. As a result of such observations Schwann reached the broad conception that, like the plants, an animal’s tissue is made up of certain elementary parts which are either knitted together by, or formed by a metamorphosis of, these elements. Both Schleiden and Schwann defined the cell

as a small chamber consisting of a definite membrane with a fluid content. Of this small entity they considered the membrane to be the essential part. According to Schwann's view, the cell was akin to an organic crystal, and was capable of being evolved by some special form of crystallisation from the organic mother liquid. These views underwent modification, especially in the protoplasm theory of Max Schultze. Schwann had noted that the vegetable cell contained besides its sap a soft, transparent and finely granular substance to which he applied the name of 'Pflanzenschleim.' To this Mohl gave the name *Protoplasm*. The interior of young plant cells was found to be completely filled with this substance or protoplasm, which in older cells took up fluid which eventually accumulated to form vacuoles. It was further found that this protoplasm was capable of exhibiting movements. Cohn and other observers, in their investigation of the lower Algæ, noticed that the protoplasm at the moment of reproduction retracted from the cell membrane to form a spore which escaped through the cell membrane to lead an *independent* life as a motile organism, but *without* a cell membrane. Similarly Kölliker and others noted that there were animal cells in which a special cell membrane could *not* be demonstrated. The question therefore arose, were these elements *without* visible membranes to be regarded as genuine cells? The answer is *yes*, and the significance of the term 'Cell' underwent consequently a profound modification. The granular ground substance of certain animal cells was observed to exhibit movements similar to those to be seen in plant cells, and the term *Protoplasm* was likewise applied to the

ground substance of the animal cell. We have reached, therefore, the standpoint that in every cell the physical basis is the substance known as Protoplasm. The study of unicellular organisms served to confirm this protoplasmic theory, and the protoplasmic substance as met with in simple animal cells was frequently termed *Sarcodæ*. Sarcodæ and Protoplasm are, however, identical substances. Further research led to the conclusion that the cell membrane was a structure of subsidiary importance. Whilst the plant-cell protoplasm generally possesses a containing membrane, it is not a necessary vital constituent of the cell, inasmuch as free living derivatives of the vegetable cell may lead a perfectly independent existence without it. As a matter of fact, many unicellular organisms, whether plant or animal, possess no such membrane, and go through the phases of their life as naked masses of protoplasm. The cell membrane is *not* an essential constituent in an animal or vegetable cell. The cell must therefore be regarded as a mass of substance—protoplasm—endowed with the properties of Life, and to this the term of *elementary organism* was applied. The early description of the cell as a closed chamber must therefore be abandoned. Our knowledge of the nature and properties of the cell has been greatly increased in recent years, and the formed body or *Nucleus* which has been so universally shown to exist in the substance of the protoplasm, is now generally regarded as an integral and indispensable component. The older definition of the cell as consisting of a closed chamber with a fluid content was replaced by its definition as a mass of protoplasm. And now the cell-unit is generally

regarded 'as a mass of protoplasm which contains in its interior a specially differentiated constituent—the *Nucleus*.' Altmann has endeavoured to prove a still lower grade of individuality than the cell, consisting in certain granules which are to be found in the homogeneous cell protoplasm. Altmann regards these granules as the true elementary organism and the seat of vital phenomena. To these granules he has accordingly applied the name of *Bioblasts*. This view, however, is not one that is generally accepted, and the great majority of observers reject this definition. The cell essentially consists of a ground substance, *Protoplasm*, in which is contained a differentiated body known as the *Nucleus*. Both are integral conditions of the Life of the Cell. The protoplasm, when removed from the nucleus, is not capable of independent existence; and the nucleus, when removed from the protoplasm, cannot maintain its life processes. The one requires the other, and it is by their joint action that the functional activity of a cell is made possible. As already stated, the cell membrane is not an essential constituent, as many cells are to be found which carry on all the processes of life without this adjunct. Enough has been said to indicate the influence of the Cellular Doctrine on physiological as well as on pathological research. The cell is the central fixed point, no matter how wide the circumference of Life be drawn.

LECTURE II.

The Cell—Reproduction—Differentiation of Cells—Cell Functions
—The Amœba as an Example of a Typical Cell—Temperature
Requirements—Amœboid Cells among Plants—Cell Colonies
and Cell Division—Types and Modifications of Cells.

In our first Lecture upon the Cell as the Unit of Life we referred to the development of Physiological Research, which in so many different directions had led forward to the centre of present-day inquiry—the Cell. We noted how not only in the present, but in the future, the Cell and its problems occupied, and would occupy, the attention of Biologists, not merely in its structural, but likewise in its functional, properties. With regard to physiological method and inquiry, we found that the nature of the inquiry determined the special method to be employed. The problems were of so complex and varied a character that the methods employed must themselves be of a varied nature—that if we failed with one method we must try another, or, in other words, endeavour to find the key which would fit the lock. We touched on certain great theories concerning the processes going on in the world around us. The Atomic Theory, the doctrine of the Conservation of Matter and Energy, as well as of the forms of energy residing in Matter, whether in an Actual or Potential condition, as well as the attitude adopted by

observers towards Life and its Vital processes, notably in the instance of the Theory of Evolution. We said that for the purpose we had in view—viz. of experimental research—we must seek to explain the manifestations of Life on the mechanical principles of Chemistry and Physics. In saying this there was no intention to suggest that we would thereby satisfactorily explain what ultimately *Life* in itself was, or, in contradistinction to the Vitalistic Theory, explain the cause as well as the effect on a materialistic basis. The mechanical is, however, for scientific observers the one safe and sound working hypothesis. With its aid we endeavour to find out how much can and how much cannot be explained on a purely natural basis. Every thinking person will acknowledge how inscrutable many of the vital problems appear to be, and how often one is baffled in the attempt to reduce things to a Mathematical Formula. Science is, however, constantly renewing its Youth, and can only find satisfaction in a reasoned explanation of phenomena—in a harmony that must rest at the basis of things. We have to push on our analysis of Phenomena, whether in animate or inanimate Nature, impelled by the instinct which is so strong within us, and to seek for Causality and the reign of Law in the Natural World. This is, and must be, the Laboratory Spirit. It does not, and I am sure need not, exclude the postulates which we are able to draw concerning the World in the light of the one real thing to us—our consciousness. *Cogito ergo sum*. We pointed out how observation had led us to regard living matter, whether simple or complex, as consisting of certain elements or of multiples of the same which build up the

fabric of the Plant and the Animal. Our analysis had brought us down to a Unit, which was the smallest indivisible element at present known to us capable of manifesting the essential functions of Life—viz., Nutrition, Growth, Reproduction, Motion, and Sensation. This unit was a speck of protoplasm harbouring a formed body, the nucleus. To this in the modern acceptance of the word might be applied the term *Cell*. We shall have occasion to keep this definition constantly in mind in the course of our subsequent considerations. At the same time we have to recognise that whilst in the independent or free cell we have all that this definition implies fully expressed, and that whilst from it we obtain a valuable morphological conception of the Unit of Life, the cell itself is constantly undergoing changes. But there is always a moment, a central one in its life history, at which we can test it and find it to meet the requirements we have postulated for the living unit. Amongst these we have to note a cardinal property—the capacity of reproduction. A nucleated cell reaches a climacteric stage when it divides, multiplies, reproduces itself. All growth or development in the realm of Life means cell multiplication, with a transmission of Life and its properties to the succeeding generations of cells. We have, however, to bear in mind that the cell may undergo modification, especially of a functional character, when it exists not as an independent unit, but as one of an aggregate of cells, as, for example, are to be met with in a tissue of the human body. In the latter case the cell subserves not simply the requirements of its own Life; it has to minister to the requirements of the whole system of

cells of which the organism is made up. Whilst, therefore, the structural unit may remain as stated, we must be prepared to find, and do as a matter of fact find, important functional modifications in the unit. This usually takes the form of some one highly specialised function. Thus, for example, the cells frequently produce substances which, if it is difficult to regard as essential for their own individual requirements, are obviously most useful in the general economy of the whole cellular system—*e.g.* we have as a highly specialised function the production of digestive Ferments or Enzymes by the cells of the digestive tract, and the manufacture and secretion of Mucin by the cells of mucous membranes. And if we regard plants, we find, besides the production of ferment bodies, a storing up of reserve foods which are of the greatest value to the seed and to the plant as a whole. This need not, however, disturb our morphological conception of the Cell as the essential and derivatory Unit of Life in its simple or in its complex attributes. In the simple free floating cell we find a unit displaying the basal phenomena of Life. It absorbs food, it undergoes a period of growth, it exhibits movement and a response to stimuli. It reaches a climax, a crisis of reproduction, after which it passes through a period of decay to death. In such a simple cell there may be said to be a balance between the various essential functions. In its complex and aggregate condition modifications may and do occur, and this is, as I have said, generally expressed in the form of functional specialisation. The physiological balance is disturbed, and whilst the essential vital expressions remain the same, some one function predominates and obscures the others—*e.g.*

glandular secretion, muscular contraction, oxygen transmission, nervous impulses, and so on. How far this specialisation of function may be carried we see in the study of Embryology. Note that marvellous property which leads not only to the transmission and permanence of the type, but to the transmission of peculiar hereditary properties which may at once express themselves in an immediate, or may first come to the surface in a subsequent, generation. Could one find any more striking example than this of the potentiality of Life, residing in the tiny speck of protoplasm, so sensitive, so fragile, and yet endowed with all the possibilities of which Life, in its highest and noblest manifestations, is capable? It will be essential before proceeding to discuss the vital properties of the cell to devote our attention in the first instance to the cell as it presents itself to us in Nature, and to a consideration of the structure on which all vital functions rest. That is, to understand something of the morphology before proceeding to the physiology of the cell. I think we can best do this in the first instance by focussing for the moment these two points of view—the morphological and physiological—in some typical examples drawn from the simplest forms of Cellular Life. Having considered these primitive cells in their structure and in their function as a whole, we will then separate the two points of view and follow more closely the morphological basis into the sphere of function, as seen in a variety of cells of vegetable and animal origin. Let us then commence with a short consideration of a typical free living animal cell, selecting for our purpose an easily observed example—the *Amœba*. If we examine an *Amœba* in a drop of water

under the microscope we observe a small viscid mass of substance with a visible contour and differentiated from the surrounding water by its higher refractile power (Plate, p. 42, *a*, and fig. 1, p. 36). It usually exhibits movement, stretching out from its surface spontaneously short extensions, known as pseudopodia. A confining wall or cell membrane is not seen—the body of the organism is naked. The surface layer of this jelly-like mass of protoplasm is free from contained bodies or granules and has a glassy transparent appearance. This surface layer is known as the Ectoplasm. Within it we have the greater mass of the protoplasm, which is duller in appearance and is not homogeneous. It contains granules, and constitutes the so-called Endoplasm of the organism. In this layer of protoplasm we likewise note the presence of a differentiated body—a Nucleus. The whole cell is continually changing its form, and hence the name *Amœba*, as well as the term *amœboid* movement, applied to such movements in general. The surface of the cell bulges at one point, and a projection appears which becomes larger and larger as more protoplasm flows after it, and we get a streaming of the cell substance from the centre to the periphery along the line of the projection or pseudopodium. The *Amœba* may quickly produce several of these pseudopodia. This stretching out of a foot is followed by a retraction, and the protoplasm flows back centripetally and the *Amœba* returns to a rounded form. There is therefore a phase of expansion and contraction in the movement. We find, also, that this *amœboid* cell may contain unabsorbed and undigested substances, such as granules of sand. In

the case of a young cell we note that it becomes larger, that there is a process of growth whereby it approaches the size of the mother cell. The cell increases in bulk, it grows. For this as well as the other of its life processes it requires food, and inasmuch as it does not possess a containing membrane the substance of the cell is able to come into direct contact with solid particles of food and to enclose them in its interior. For example, the *Amœba* crawls up to an alga cell by means of its pseudopodia and then surrounds the alga with them. The protoplasm next flows round the vegetable cell which becomes incorporated into the substance of the *Amœba*, becoming at the same time surrounded by a thin watery mantle, which is known as the Nutrient Vacuole. We have therefore already noted in this free living individual cell some of the cardinal functions of Life—viz., Growth, Spontaneous Motion, and Nutrition. These have their parallel in the free cells of the animal body—the leucocytes—which are likewise motile wandering cells and are capable of ingesting solid substances from the blood. The food thus obtained is dissolved or digested and the suitable elements assimilated for the purpose of building up and supplying energy to the organic substance of the cell. The undigested remainder of the food remains in the so-called nutrient vacuole of the *Amœba*, and is got rid of as follows. In the course of the perambulations of the *Amœba*, the vacuole comes close to the surface, and the then surface layer of protoplasm gives way very easily, so that the contents of the vacuole flow out of the cell. We have here, therefore, the simplest manifestation of the process of excretion. When a cell in the course of its growth

reaches or begins to exceed the normal size, it divides into daughter cells. The simplest form of this process of multiplication is a direct division of the cell. In the *Amœba* (fig. 1) we note that the rounded nucleus becomes elongated, *a*, then constricts in the middle, *b*, and divides into two new nuclei which assume the rounded form, *c*.

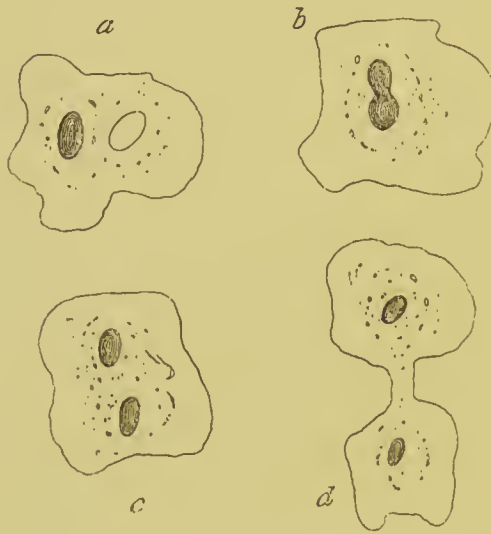


FIG. 1.—Diagram of the stages in the division of an *Amœba*.

The general mass of the cell protoplasm likewise divides and becomes ruptured between the two nuclei, *d*, with the result that two new complete *Amœbæ* are formed. We have here the phenomenon of reproduction in its simplest form—viz., a simple fission of a mother cell with consequent multiplication

or a production of two fresh units. It is of importance to note the participation of the nucleus in the process—the nucleus divides as well as the protoplasm, it is an integral sharer in the process, and it remains as a nucleus and as an integral part of the new cell. This attracts our attention and suggests that an important part is played by the nucleus in the life of a cell. We shall have occasion to return to, and to make fuller mention of, this fact as we proceed further. Suffice it to say at this moment that there has been

a transference of *nuclear* as well as of protoplasmic substance to the daughter cells. The living cell also absorbs oxygen and gives off carbonic acid. Oxygen is a necessity for the life processes of the cell, and its absorption, followed by the excretion of carbonic acid, represents the beginning and the end of the process of Respiration. Oxidative processes are essential to Life. How does the *Amœba* behave in this respect? In the first instance, we note that if oxygen is withdrawn the amœboid movements of the cell become paralysed. If the oxygen is completely driven off by hydrogen gas, the *Amœba* gradually ceases its movements and ultimately dies. With the withdrawal of oxygen life ceases—it is one of its general requirements.

A cell is not inert, but is highly sensitive, to the influence of its physical environment, and to none more so than that of Temperature. In fact, the full exercise of the functions of Life depends on certain external conditions of temperature. An *Amœba* if exposed to a temperature of 40°–45° C. (104°–113° F.) dies, and if the temperature is sufficiently lowered we have likewise a paralysis, and, finally, an extinction of the vital processes. Somewhere between these two points lies a temperature which is accurately suited to the requirements of the cell—an optimum temperature. This is a typical instance of the influence of *environment* on vital functions. In death we have a granular break-up of the protoplasm, and a subsidence into unorganised *débris*, which in its turn finally disappears from our view. The normal organism is in a condition of struggle with its environment, and within certain limits it is able successfully to carry on the

normal phenomena of Life. If, however, the external conditions vary, or are varied beyond a certain mean, the organism reacts accordingly. This process, whereby a reaction to changed external conditions occurs, is known as stimulation, and the agents which produce such a reaction are known as stimuli. We have already spoken in our first lecture of Excitability or Irritability as a characteristic property of living matter. How is it with the *Amœba*? The immediate presence of a cell of an alga is sufficient stimulus to set the *Amœba* in motion; it creeps up to its prey. If we add to water containing *Amœbæ* a 1-2 per cent. solution of sodic chloride or a dilute solution of potash or of hydrochloric acid, or if carbonic acid gas be added, we find that the pseudopodia are retracted, and the *Amœba* assumes a rounded form. We have a phase of Contraction. If we replace the carbonic acid by oxygen, the *Amœba* resumes its active movements of expansion and contraction. In the absence of oxygen the *Amœba* shrinks—it contracts; in the presence of oxygen it expands. From the above it will be seen that a stimulus may have a twofold effect: it may excite or it may depress vital function. In fact, one and the same stimulus may combine both phases—first, exaltation and then a succeeding depression, as is seen in the action of heat and also of anæsthetics. The stimulus of a mechanical concussion or blow will cause the *Amœba* at once to cease its pseudopodial motion, and the same effect is produced by the vibrations of a tuning-fork in its neighbourhood. As already noted, increase in temperature up to a certain point increases the activity of the *Amœba*, till at 45° C. its movement ceases; and on the

other side, say at zero, its movements likewise cease—its protoplasm passes into a phase of rigidity in each case. We may, therefore, distinguish as regards the exercise of vital functions a maximum, a minimum, and an optimum condition of temperature, and this applies also to other external agents. If the minimum or maximum condition of temperature, or of any other condition of Life, is exceeded, we have a depression of vital function which may lead to death. And, as we have seen, an external stimulus may either excite or depress; this will depend not only on its quality, but on the degree of its action. It must be borne in mind that a stimulus may be of a normal character, and may be necessary for evoking the vital functions—*e.g.* the stimulus of the presence of food on the amœboid cell. When the organism reacts normally to such stimuli as come to it from its environment it is said to be in a condition of Tone, and as regards temperature such a condition would be described as Thermotonus. The amœboid cell consists of a ground substance, protoplasm, which is essentially a chemical substance consisting of proteid or albumenoid matter. In this during life chemical combinations occur, which are again broken up. The decomposition products are discharged from the cell, and new combinations are formed by the assimilation of the Elements of the Food. We have, therefore, continually going on in the living cell processes of association and dissociation, and these combined processes have been summed up in the one word *Metabolism*. Upon this intracellular metabolism the exercise of the vital processes depends. The process centres itself in the complex chemical compounds known

as the proteid bodies, and these form the essential basis of Life in the *Amœba* as well as in other cells. We have, therefore, in our short survey noted that all the essential phenomena of Life are to be observed in a simple unicellular free living organism—the *Amœba*; that the structure on which these vital phenomena rest consists of a ground substance, the protoplasm, with a contained body, the nucleus, and that there is no differentiated cell wall or membrane; that this simple cell is capable of full vital activity; that it seeks, absorbs, digests, and assimilates food, and excretes the undigested remainder; that in it there is a complete cycle of assimilative and dissimilative processes, characterised by the generic term Metabolism. This process is essentially a building up of the organic tissue of the cell along with the acquisition and utilisation of Energy. The results are the vital expressions we noted—viz. increase in cell substance or growth and active movement, till a phase in the life history of the cell is reached—the reproductive—in which, by the simplest method of fission or cell division, a multiplication of the living unit results. Two daughter cells appear, each sharing protoplasm and nucleus, and evidencing the phenomena associated with their presence in the mother cell. We have also had an indication of the essential character of the respiratory process—that vital function intimately depends on the presence of oxygen—its withdrawal lowering and its absence inhibiting cellular function. We have also found in the protoplasm of the *Amœba* one of the cardinal features of all living matter: an excitability, a response to stimuli; that the living cell is in responsive touch with its environment—

i.e. with the external world in which it may be so truly said to live and move and have its being. The stimuli that reach it may be of a normal or an abnormal character. Normally they may attract the cell towards its food; or they may be of the nature of heat stimuli, which under a given optimum condition evoke the highest capacities of the cell. If, however, we have unfavourable conditions, such as an unsuitable temperature, a lack of oxygen, the presence of unsuitable chemical substances, or the undue action of certain physical agents, the phenomena of Irritation occur, evinced in a condition of momentary exaltation or transient or permanent depression. The cell under such conditions usually undergoes a visible alteration, summed up in the phenomena of contraction. In this simple amœboid cell there is, therefore, a response to stimuli, and the stimulation may be normal or abnormal, either evoking a condition of *Tone* or passing gradually away from the optimum to the maximum and minimum conditions of the Life of the Cell, and resulting in Irritation, Depression, or ultimately Death. We have, therefore, noted in the Amœba a structural unit evincing all the essential functions of Life—viz. Nutrition, Growth, Motion, Reproduction, and Sensation.

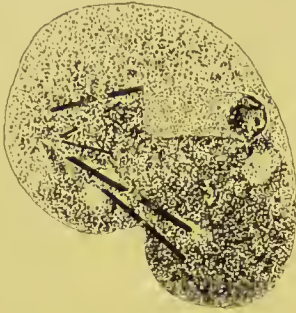
We find likewise amongst the plants, cells which approach in resemblance to the Amœba. We may mention the Slime Fungi, or Myxomycetes, consisting of a translucent protoplasm and known as the Plasmodium (fig. 3, p. 45). This protoplasm is capable of a pseudopodial motion and of a response to stimuli, whilst in the animal body we have already noted the wandering cells

42 THE CELL AS THE UNIT OF LIFE

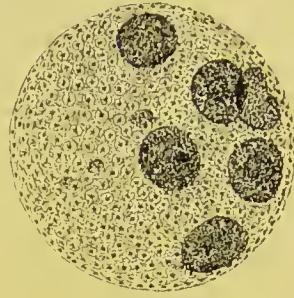
or leucocytes exhibiting likewise all the essential phenomena of Life as above indicated.

In the course of evolution these simple conditions cease to exist, although the basal phenomena remain the same. The cells become united, serving, as we have already observed, not only individual, but likewise general requirements of the aggregate in which they find themselves. This finds its expression in the congeries of cells as exist in the tissues of the plant or in the organs of the animal body. Modifications in structure may be the result, though what are typical properties in the free cell are likewise typical properties in the fixed cell. It will be useful in the first instance to consider shortly under what conditions we meet the cells when called upon to fulfil the higher conditions of Life and how far we are able to trace, even in the most complex form, the simple structures we have found wandering freely as independent units of Life. Is this Cellular Physiology of universal application? The cell is the elementary organism—it is the simplest grade of the individual. The conception ‘individual’ is not always an easy one to define. I think our best test is the physiological one of self-conservation and the ability to exercise the functional attributes of Life, which we have just discussed in connection with the unicellular organism. In the meanwhile, our general survey at this moment is directed towards tracing the cell as it presents itself to us in Nature. We have already noted the free, living, independent cells, such as the *Amœba* in the animal, and the Slime Fungi in the plant, world. The Bacteria form a typical example in the vegetable world of independent cells, which, however, in

PLATE.



a. Amæba



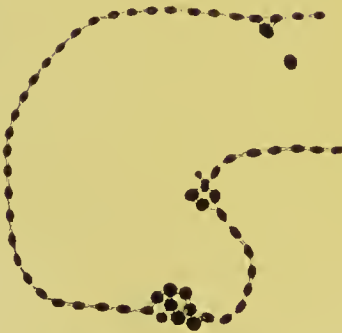
b. Volvox



c. Diplococcus



d. Bacillus



e. Streptococcus




f. Staphylococcus

T.P. Collins

contradistinction to the amœboid organisms, possess a cell membrane. In the course of multiplication the bacterial cells may separate, or may remain united in large or small clusters of cells. Thus, we may have a grouping in pairs (Plate, *c*), in fours or tetrads, or in multiples of the same, as in the case of the *Sarcinæ*. They may likewise appear as long or short chains, having a thread-like appearance when the cells are rod-shaped (Plate, *d*), or resembling a string of beads if the cells are spherical (Plate, *e*). Or they may hang together in irregular bunches resembling somewhat clusters of grapes (Plate, *f*); or, if the cells are curved in shape, a spiral or corkscrew appearance is produced. In all these aggregates, however, the cells retain their independent physiological functions, and each member is capable of an individual existence and of self-multiplication, or, in other words, of self-conservation. I cannot, perhaps, illustrate this better than by citing the method originally employed for sifting out these vegetable cells as existing in mixtures of various forms. This was effected by a process of dilution—viz. the transference of a small portion of the original natural mixture to a flask of nutrient fluid. In this way a preliminary dilution of the mixture of organisms was brought about—*i.e.*, there were fewer cells in a larger quantity of fluid. This process of dilution, if repeated from flask to flask, resulted eventually in an enormous dilution of the organisms until a stage was reached when perhaps only one cell was introduced to the penultimate and none to the ultimate flask. In the flask, the last but one of the series, there results a copious, unadulterated growth or self-

multiplication of the cell in question, which reaches uncountable numbers.

The powers of self-conservation and reproduction in such an individual cell can perhaps be most strikingly illustrated in the case of the Anthrax Bacillus. One active cell introduced into the body of a susceptible animal is capable, in a few hours, of a reproduction which reaches millions, and of producing the death of its host. Each individual is a complete physiological unit.

We next note in Cell Life the aggregation of the Units into Colonies, as, for example, amongst the Algæ and Infusoria. Thus we find cells which secrete a gelatinous substance within which the cells, after reproduction, remain united together to form a *Colony*. The cells contained in the imbedding substance may be separate from one another, they may be connected by a fine thread of protoplasm, or they may be in contact. The colony may be temporary, the cells eventually separating, or it may be a permanent one. We may take three examples from the group of the Algæ. In the first instance the cells are at a little distance from each other [thus: ○○○○○○○○]; in the second instance the cells touch at one point of the surface [thus: ○○○○○○○○]; or, in the third instance, the cells are united at one surface [thus: ◻◻◻◻◻◻◻◻]. We may also have a colony united on a common stalk [thus: 

We now come to another phase in which the multicellular connection is still more intimate. The cells may

fuse together, or, whilst remaining differentiated, are at the same time intimately connected. The congeries of fused cells are known as *Cænocytes*. We have, for example, the Slime Fungi, or Myxomycetes, which are an aggregation of a number of naked cells, which have combined to form a plasmodium. This plasmodium is multi-nuclear (fig. 3). The genuine Cœnocyte does not occur by the fusion of a number of individual cells, but by production from a single mono-nuclear cell. The nucleus divides into a number of nuclei—2, 4, 6, 8, &c. The protoplasm does not divide correspondingly, it in-

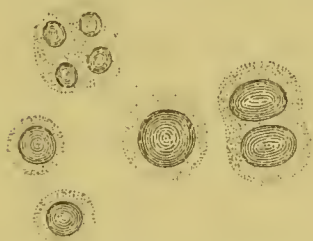


FIG. 2.—Diagram illustrating the cells of Proto-coccus.

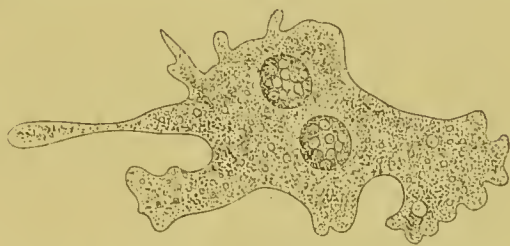


FIG. 3.—Part of the Plasmodium of a Myxomycete, with two nuclei. (After Sachs.)

creases mainly in bulk. Amongst algæ and seaweeds such forms are found, with the whole colony protected by an external membrane. Each nucleus can form the potential focus of a fresh cell.

Passing from the *Cænocytes*, we come to another phase of this more intimate cell connection, in which the individual cells are distinctly differentiated from one another, though at the same time in close juxtaposition and aggregated into a more or less uniform organism. In this class may be reckoned the more highly organised plants and animals with varied structures and organs. We have the various steps in the ladder from the simple

moss plant to the highest developed flowering plant, and from the simple Hydra polyp to the vertebrate animal with its organs and tissues. We meet with a specialisation of function and a division of labour.

Let us trace this differentiation in the plant body. In some of the simplest forms we have a closely packed external layer of cells for protective purposes, as in the simple seaweeds. In the higher plants this takes the form of a protective tegumentary system, consisting of thickened cells. Further, a formation of cells and vessels for transport occurs—a conducting system, known as the vascular bundles, and generally consisting of two parts: the woody tissue for the ascent of water, and the bast tissue for the transference of metabolic products. We have further a differentiation of cells into a skeletal or supporting system for the plant, as in roots, stems, branches, and leaves. And, finally, we have a differentiation of groups of cells for metabolic purposes, such as the formation of glandular tissue, and specialised organs such as the Chlorophyll bodies. In the plant, therefore, we meet with four main cellular differentiations: 1, the Tegumentary; 2, the Conducting; 3, the Supporting; and 4, the Metabolic System of Cells.

We have further a compound of organic individuals with a high degree of independence as regards its parts. Thus the fractional individual may be separated from the whole without losing its power of self-conservation by this isolation, and this fraction may by multiplication renew the original compound individual—*e.g.* the Cœlenterata and the worms. The fractions in such instances may be similar or only slightly different. The corals are

an example of a similar aggregate of individuals. The factors of the aggregate, on the other hand, may be markedly different, as, for example, amongst the Siphonophora, with differentiated swimming bells, nutrient and other organs. We have a series of intimately connected but differentiated *persons*. The aggregates, however, are made up of and spring from cells, and remain together in virtue of the affinity of their common origin, and this likewise ensures the permanence of the type. We have such an affinity of cells represented likewise in the fertilisation of plants. We likewise find in Nature associations of cells of different origin which by their association mutually benefit each other, *e.g.* the Symbioses of Fungi and Algæ. The lichens which are epiphytic on trees, rocks, &c., consist of two distinct plants, an alga and a fungus, closely united to form a kind of Thallus, each with a different metabolism, but mutually useful. The chlorophyll-containing alga can assimilate carbonic acid, and form therefrom carbohydrates. The non-chlorophyll-containing fungus cannot do this, but shares the formed carbohydrate with the alga. The fungus condenses aqueous vapour, and dissolves *débris* which can be utilised by the algæ cells, and likewise attaches the compound individual to the substratum. The fungus cell cannot produce an alga cell and *vice versâ*, though they unite to form a mutually useful partnership. This is of great interest, and I may be allowed to quote Sachs, the distinguished botanist, on the subject:—‘It is apparent that the chlorophyll-containing algæ in the lichen are active as assimilative organs in the same way as chlorophyll-containing cells on the surface of a green twig or on a

leaf, furnishing nutritive material to the lichen, whilst, on the other hand, the necessary ash constituents are supplied to the algæ cells by the fungus. By means of this convivium the lichens are rendered independent of an organic substratum. Whilst all other fungi are parasites or inhabitants of humus, the lichens can settle on a purely mineral soil, even on the surface of crystalline rocks, inasmuch as they are rendered independent by the algæ they contain. They are capable of decomposing the inorganic substance of rocks, *e.g.* granite, in order to obtain, in a similar fashion to the roots of the higher plants, those mineral substances which their chlorophyll-containing cells, the algæ, require for assimilation in their tissues. These fungi, through their conjunction with given algæ, in order to obtain nourishment from them, gain a *freedom* in the choice of their place of abode, which is not at the disposal of any other fungus.' We have other instances in the vegetable kingdom, such as in the Kephir—the symbiosis of a yeast and a bacterium. We have likewise the root tubercles of the Leguminosæ, in which there is an association of a bacteria-like organism with the plant root cells. The fungus probably fixes free nitrogen for the plant's use, and obtains carbohydrates from the plant. We may also find a symbiosis of an animal cell with a unicellular alga, *e.g.* amongst the Radiolaria, which may contain alga cells in their cell protoplasm, which multiply, but are retained for the use of the Radiolarian. In many Actiniæ one finds algæ cells inside their ciliated cylindrical intestinal cells, and multiplying in these cells. It has been suggested that they may be mutually useful in the exchange of carbonic acid

and oxygen. It likewise affords protection to the alga, the metabolic products of which may, no doubt, prove useful to the animal. As it has been well put, we have here to a certain extent the same circulation of matter which occurs in Nature between the animal and vegetable worlds, focussed in the symbiosis of a plant cell and an animal cell. And besides this symbiosis we may have an invasion of a cell territory by a foreign cell to the detriment of the cells attacked. This is known as Parasitism, and a striking example of this is the causal relation of bacteria to infectious diseases. The vegetable cell is favoured by its hosts, but produces usually exquisite poisons, with consequent degeneration of the tissue cells and fatal effects on the centres of Life.

The closer relation of cells in a complex organism may be brought about in various ways, and in their description I will follow Hertwig.

I. We may have a mutual influence of the cells by direct contact of their surfaces. Thus non-membranous cells by close contact may communicate stimuli to one another.

II. We may have a connection of individual cells by protoplasmic threads or a series of intercellular bridges. For example, in species of *Volvox* we find cells, each surrounded by a gelatinous mantle, and each united with five or six neighbouring cells by fine strands of protoplasm (Plate, *b*). It has further been demonstrated on a number of plant objects that fine pores occur in their cellulose membranes, through which connecting threads of protoplasm pass (fig. 4). And similar appearances can be noted in the connective tissues of animals. These

intercellular connections continue to be the subject of further investigation; the subject is naturally a difficult

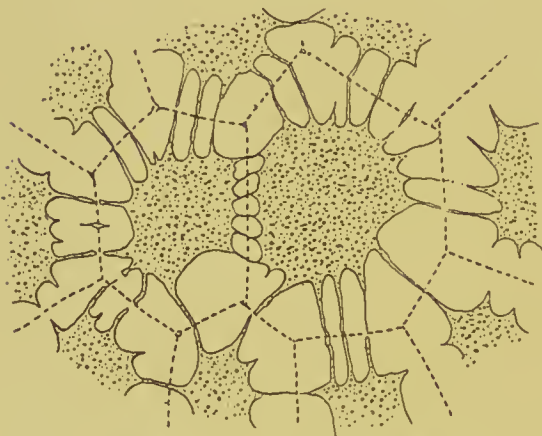


FIG. 4.—Diagram illustrating the strands of protoplasm connecting the cells of a plant. The dotted lines indicate the cell-membranes. (After Gardiner.)

one for demonstration. The connections may serve for transference of stimuli or for transport of substances.

III. We may have a connection of cells by nerve fibres. In this way a direct connection between parts

widely separated is brought about, and in this way stimuli are transmitted.

IV. We may have a connection of cells by the fluids circulating in the organism. For example, in plants by the upward circulation of the nutrient sap from the roots; and in animals by the lymphatic and blood circulations. The normal constitution of the blood depends on a large number of organs. A disturbance in one organ varies the constitution of the blood, and in this way influences the metabolism in a number of other organs. We have now got so far that we may say that all living structures are cellular structures or their derivatives. Plants and animals are aggregates of cells produced by division of a common mother cell, and remaining in intimate connection. The originally uniform elements pass through a long series of changes, which we term the developmental process of the

organism, and separate into the most different, but at the same time harmoniously working, parts of the plant and animal body, through the influence of internal and external causes, which we cannot attempt to trace in this present course of lectures. The external and internal factors of organic development constitute a very difficult chapter in Biology, requiring individual and separate treatment.

In the complex cellular organism we note a physiological division of labour—thus in the plant we have special chlorophyll-containing cells—with specialisation of function we have corresponding changes in structure, *e.g.* muscle, gland, nerve, &c. Similarly functioning cells lie usually in groups, and these are called tissues, *e.g.* muscle tissue, &c. The cells become dependent parts of a higher and supreme living unit, and in the process the individual cell units cease to be capable of *independent* existence—removed from their connections they die. The independence of the cell as an elementary organism is lessened, the cells acquire specific functions, and react to stimuli with their specific energy.

We have said enough to indicate the biological importance of the Cell—all cells are derived from cells, all living structure is made up of cells, and the most complex organic individual has had its origin in a cell. And in the complex organism there is a solidarity betwixt the cells, and each may influence the other. The changeableness of the tissues under such internal or external influences belongs to the domain of Pathology, and as we have a Cellular Physiology, so we have a Cellular Pathology.

In the plant its structure rests mainly on the properties and action of chlorophyll. The energy of the plant is directed to the formation of organs, which by a very small thickness will easily submit themselves to the action of Light, and which at the same time will present as large as possible a surface of chlorophyll-containing tissues to the air. The whole endeavour of the plant is therefore to give its structure a form which is directed *outwards*, and hence the numerous unfoldings of plant life which delight our eyes. In the animal the differentiations are directed *inwards* in the form of organs and tissues.

The surroundings of a plant influence its cellular structure, especially in two main directions—viz., as regards mechanical and circulatory tissues. In aquatic plants mechanical supporting tissue is not required; they float in the water. In land plants their aerial parts require and develop a supporting tissue or framework. As regards a circulatory tissue, in the aquatic plants such as seaweeds, they are surrounded by water, and do not need to develop special circulatory tissues; their cells can take up water and dissolved nutrient matter in a direct fashion. This is not the case with the terrestrial plant. It requires roots to absorb water and salts for all the other cells of which it is composed, and stems with branches and leaves to furnish necessary food for the roots and other parts. To facilitate this exchange a flow of sap is necessary by means of vessels. We have, therefore, a combination of mechanical and circulatory tissues in the terrestrial plant.

As regards the *animal* cell, in contradistinction to the plant, it takes up preformed organic substances, and in

the non-membranous cells solid matter can directly enter the cell substance, or in the membranous cells soluble organic matter can diffuse into the interior through the membrane. In the animal tissues the cells are very closely packed and intimately related. And the functional organisation is one that is directed *inwards*, and is built up round a gastric or digestive tract, so that the nutriment passes from within outwards. The *external* development of absorbent surfaces which we noted in plants becomes an *internal* one in the case of animals. Between this digestive tract and the outer tegument we have, therefore, a marked development of cellular tissues and organs. And in the formation of the organ we have an abstract of its developmental history. And whence do these cellular complexes arise? They all start from a physiological unit—a cell, bearing with it not only the essential vital activity, but all the acquired and inherited peculiarities of its predecessors.

We started with the purpose of seeing how far a cellular physiology was of general application. We found that single cells existed in a free condition, which exhibited all the essential properties of Life, that they consisted of certain structural elements, that they reproduced themselves, and remained true to the ancestral type. We found that in tracing Life, not merely in its simple but in its complex manifestations, a cellular structure was to be found, or one that had proceeded from cells. That, when we analysed further, the activity of animals and plants was found ultimately to rest on tissues or organs of cellular structure. In the simplest free-living cell we found a complete individual, structurally and functionally.

A congerie of such cells, each of equal value, gave rise to a *simple* tissue. A union of cells of different degrees or qualities gave rise to a compound tissue or organ. And a conjunction of such organs represents a higher animal or Man. In all cases, despite varied specialisation or division of labour, the cell remained the elementary organism. From the cell all Life starts, and the object of Life is to produce that remarkable product a SPECIES CELL, which, in virtue of its hidden hereditary and imprinted characteristics, is able to renew the complex life, and to transfer afresh the stamp which Life puts upon matter.

LECTURE III.

The Structure and Chemical Composition of Protoplasm—Physical Characters of Protoplasm—Cell-structure—The Nucleus—Nutrition of the Cell—Ferments.

In the previous lectures we have endeavoured to indicate the trend of physiological inquiry, how past research has led to the present cellular conception of living matter, and how investigation was now so largely directed to the study of cell life, whether in a physiological or in a pathological sense. Embryology, too, that all-important branch of investigation, has advanced in the direction of tracing structure and function to their origin from the primitive species cell. Forwards or backwards, so far as our analytical methods reach, it is always the cell, whilst in the future the most difficult vital problems remain to be attacked in the cell itself.

We proceeded to inquire into vital phenomena, and found these to rest, whether in their simple or complex expressions, upon the single or the combined action of cells. The simple *Amœba* and the tissue cell showed the same basal phenomena of life, and everywhere we traced cellular structure and activity. All life is derived from the cell, all function rests in it. We therefore felt justified in the statement that the most valuable biological

doctrine which our present knowledge has enabled us to reach is the cellular conception—of the cell as the essential Unit of Life—whether free in an isolated living form and in physiological balance or fixed in connection with other cells and showing functional specialisation. The cell, reduced to its simplest terms, is a speck of protoplasm with a contained body, the nucleus, and possessing all the evolutionary possibilities we have already indicated. After testing the structural and functional definitions of living matter on the unicellular *Amœba* and other free-living units, we proceeded to trace and to find the cellular structure pervading all forms of vegetable and animal life, whether they consisted of simple or compound tissues or organs. Having thereby outlined the general structural and functional characteristics which have to be kept in view, we may now return to trace in more detail the structural and functional data we possess concerning the cell. A knowledge of structure is essential for the understanding of function. We must first consider what is known regarding the physical basis that renders the activities of Life possible. And as regards this physical basis we have to regard it in its morphological, physical, and chemical properties; in other words, we must consider for a moment what is known of the nature and properties of protoplasm, what is this proteid molecule built up of? We have already stated that in the modern sense of the word the cell consists of a mass of protoplasm containing a body known as the nucleus. It is true that Altmann has endeavoured to prove a lower grade of individuality as regards the Unit of Life—viz., in certain granules to be found in the cell substance, the so-called

·Bioblasts.' These constitute, in his view, the true seat of vital phenomena. This point of view has not met with general acceptance. The definition we have already given remains the best and surest one that we are at present able to formulate. We will not here dwell upon the Polar Body or Centrosome which is regarded by some observers as an essential cell constituent. Our references to the cell will be in the sense already defined. It need only be remarked here that a cell may contain not merely one, but several nuclei. It will naturally occur to you that the investigation of such a minute object as the cell must be a matter that is beset with difficulties, and of these there are only too many. We require the best optical aids for its proper investigation, and the modern improvements made in the microscope have led to advances in our knowledge of the cell. There are, however, many features which must exist that still remain on the other side of visibility. The eye is, however, capable of wonderful education in this respect, and many things become perceptible to the trained observer which escape the notice of the less experienced. Further, micro-chemical reactions are capable of further development, though the interpretation of their significance is not always easy. The various forms of anilin and other stains have been of great help in tracing structural elements in the cell, though it ought to be remembered that many of our methods modify or completely alter the condition of the cell as it was in its living or active phase. Further, the exact chemistry of the living substance is still a *terra incognita*. We do not yet possess a chemistry of the living proteid molecule. I have mentioned these things

so that we may understand the reason for the many gaps that exist in our knowledge. We will consider the protoplasm and the nucleus separately. I may repeat that the cells in the course of their subservience to the functions of Life in the Animal and Vegetable Kingdoms undergo, notably with specialisation of function, sundry and varied changes expressed in structural peculiarities. It is therefore natural that in studying the basis of the varied phenomena of life one should in the first instance seek to do so on the simplest and least complicated test objects, such as are to be found amongst young plant cells, the *Amœbæ*, and the wandering lymph cells in vertebrate animals. In such circumstances the protoplasm appears as a more or less viscous substance, which is more refractile than the watery medium in which it is examined. This ground substance of the cell has a homogeneous appearance. In it one notes the presence of minute granules (the microsomes), which may be sparse or numerous. In many instances one is able to note that the protoplasm is of two degrees of consistency—viz., a larger inner and more fluid zone in which the granules are contained, and an outer thinner and more consistent zone which presents a clearer appearance. These may be distinguished as the Ecto- and Endo-plasm. When we come to inquire into the chemical constitution of protoplasm, we find how superficial our knowledge of this substance is and that the term ‘protoplasm’ is really a morphological conception. Any attempts that we may make at a purely chemical analysis destroy its essential structure and it ceases to be protoplasm. Its generation and reproduction do not take place under laws that we

are able to follow. It is constantly being formed out of pre-existing protoplasm, and the properties which it exhibits are the result of a long education of the cell, which goes back to the very origin of things. We are not, however, justified in regarding it as a chemically uniform substance. In the cell a constant weaving and unweaving of substance must take place, a constant association and dissociation of molecular groups, a perplexing protean instability which it is impossible for us to follow. As soon as we reduce the basis of these phenomena to a condition in which we are able to examine it we degrade it from a condition of life to one of death, and a re-synthesis is impossible. We can lead the atoms we find into every channel save that of Life. There is no element in the chemical structure of protoplasm that we have not been able to find in the inorganic world. Still, when we are able to reduce the basis to chemical terms which we are able to appreciate, the unstable substance has been changed and it is no longer what it was. The essential compounds appear to be proteid bodies which form the seat of the active metabolism of the cell. The vital chemistry of the cell has, therefore, been defined as the Metabolism of Proteids. The proteid molecule contains Carbon, in association with the elements Hydrogen, Oxygen, Nitrogen, and Sulphur. And in living substance generally twelve elements are found—viz., Carbon, Hydrogen, Nitrogen, Oxygen, Sulphur, Phosphorus, Chlorine, Potassium, Sodium, Magnesium, Calcium, and Iron. The structure of proteid bodies out of Carbon, Hydrogen, Nitrogen, Oxygen, and Sulphur, leads to their designation as nitrogenous bodies or nitrogenous

constituents. They are met with in the cell in a fluid condition, and are capable of undergoing two great modifications—viz. into a more solid condition by the process of coagulation, and into diffusible forms—the peptones—by the action of ferments or enzymes. Proteid bodies are found both in the protoplasm and in the nucleus of the cell. In the protoplasm they are mainly simple proteids and phosphorus-free proteid compounds. In the nucleus they occur mainly as phosphoric acid combinations—viz. the bodies known as *Nucleins*. These two forms of substance behave differently. Thus by artificial digestion of the cell by aid of an enzyme a solution or digestion of the protoplasm occurs, whilst as regards the nucleus the nucleins contained in it remain undissolved. Desiccated proteids take up water readily; and protoplasm itself is rich in water and may contain as much as 70 per cent. The protoplasm contains various inorganic salts which are composed of some of the twelve constituents I have already mentioned—*e.g.* Chlorine, Sulphur, Phosphorus, Potassium, Sodium, Magnesium, Calcium, and Iron. Living protoplasm has an alkaline reaction. The protoplasm may further contain chemical entities, such as different forms of carbohydrates—*e.g.* starch and glycogen—and also other bodies, such, for example, as globules of fat; and of course there are the inorganic constituents to which we have already referred. We have, further, the various products of cell metabolism, to which we shall have occasion to refer subsequently, and about which it has been possible to obtain a great deal of information by means of chemical analysis. A crystal is the ideal aim of a chemist when he wishes to obtain a pure

substance for the purpose of exact analysis. And the attempt has been made to obtain crystallisable proteid substances. This has been attended with a certain measure of success in respect to vegetable albumins, and crystallisable albumins have likewise been obtained from egg albumin. These contain the essential elements Carbon, Hydrogen, Nitrogen, Oxygen, and Sulphur. The red pigment of the blood is a crystallisable proteid compound, consisting in the combination of a proteid body with an iron-containing substance, *Hæmatin*.

With regard to certain physical properties of protoplasm, the opinion is widely held that its condition is not so much that of a perfectly homogeneous fluid as that of an emulsion. The protoplasm is generally specifically heavier than water—*e.g.* the specific gravity of the *Paramœcium* is 1.25. Its specific gravity may, however, be affected by substances specifically lighter than water, such as fats and contained gas bubbles. Whilst protoplasm is generally colourless, it may assume a pigmented appearance, as is brought about, for example, by the green colouring matter of plants; and amongst the bacteria we have frequent instances of pigmented colonies or growths. We have spoken of the homogeneous appearance presented by protoplasm as observed under natural conditions. In recent years there has been a more minute study of protoplasm in its microscopical features, with the view of determining whether protoplasm is structureless or whether a finer differentiated structure really existed. The results of the finer methods of observation have led many observers to the conclusion that protoplasm possesses a

definite structure, though opinions differ considerably, not only as to the nature of this structure, but as regards the exact interpretation to be put on the observations made. We may here trace the main points of view that have been put forward. It is held by some that protoplasm consists of a very fine, thread-like network, in the meshes of which its fluid substance is contained. Or it may be compared to a sponge—the protoplasm possesses a spongy structure. The granules it contains lie at the points of junction of the threads of the net. In connection with this Bütschli remarks: ‘It is often very difficult to decide if the network structure as described by the earlier observers is actually a delicate plasma structure or if it depends on a coarser form of vacuolisation.’ Bütschli favours more the view that the structure of the plasma is of a ‘foamy’ nature. By mixing olive oil with salt or sugar and then shaking the mixture, Bütschli obtained ‘froths,’ of which the main substance was oil permeated with numerous closed spaces which were filled with watery fluid. The diameter of these spaces was usually less than the one-thousandth part of a millimetre, and they were separated by very fine oil lamellæ. If fine particles of matter were added to this oily mixture, they assembled at the meshes on the mixture being frothed. In fine froths he noted that the superficial oil-chambers were arranged in such a way that their separating walls were placed at right angles to the surface, and had consequently a parallel arrangement to one other. This layer he termed the *alveolar* layer, and he believes that this arrangement exists with all plant and animal cells. The artificially produced oil films correspond to a plasmatic framework, in the

meshes of which the cell granules collect and the superficial alveolar layers, as just described, are frequently to be observed. It may be urged against this view that whilst oil is not miscible with water, proteid matter is. Protoplasm is not a mixture of two non-miscible fluids, oil and water, but is a combination of coherent organic substances along with much water. Flemming supports another view as to the structure of protoplasm. In many living animal cells he observed fine thread-like structures in the protoplasm which were more refractile than the intervening substance. Whether they were separate or united in a network could not be determined. Flemming supposes that two substances exist—the substance composing the threads and the substance existing between them; or, as one might say, a fibrillary and an interfibrillary substance. Another theory is the Bioblast theory of Altmann, to which I have already alluded. By special staining methods he rendered minute particles visible in the cells, which he termed Granula. They may be separate or in groups, or arranged in thread-like rows. This is the Granula theory. These granula, or bioblasts, represent, according to his view, a still smaller elementary organism than the cell, and are comparable in their nature to the bacteria which are, as he would say, free-living bioblasts. The cell is composed of these granules and an inter-granular substance. ‘The bioblast is therefore the sought-for morphological unit of all organised matter.’ The bioblasts reproduce themselves by division. They are not, however, capable of self-existence outside the cell, and are probably more of the nature of protoplasmic products.

I have already indicated the appearance of the protoplasm of an Amœba and of unicellular organisms—the hyaline outer and the granular inner layer with the nucleus. In the Amœba, lymph cells, slime fungi, and Rhizopods we have naked cell bodies. In plants and animals we find generally in the former, and frequently in the latter, a membrane or intercellular substance, *i.e.* a *cell wall*. The young vegetable cell, with the protoplasm filling its interior, has a resemblance to an animal cell with its granular protoplasm and nucleus. We find frequently in the protoplasm of plants and unicellular organisms drops of fluid containing dissolved substances (such as salts, sugars, &c.). The adult vegetable cell takes up fluids which are stored in spaces or vacuoles in its interior. These sap spaces are separated by strands of protoplasm which run to, and are connected with, a peripheral layer of protoplasm lining the inner surface of the cell wall (see fig. 5, p. 65). This condition may be modified in two ways. Either the vacuoles enlarge, their walls become thinned and rupture in places, so that a series of intercommunicating vacuoles results, forming a practically continuous cell sap chamber, or the protoplasmic strands entirely disappear, and one large sap space results, which is surrounded by the thin peripheral wall of protoplasm.

We may also find an accumulation of fluid and marked vacuole formation in naked animal protoplasm, *e.g.* the Rhizopods. The surfaces thus formed are well adapted for the absorption of nutriment from the vacuole fluids. In the animal cell such appearances are rare. We more frequently meet with included and retained formed

substances, such as glycogen, mucin, fat globules, &c. The cell may in consequence have as regards its protoplasm a foamy or net-like appearance round the various aggregates of these reserve cell substances, *e.g.* the mucous cells containing mucinogen. Amongst contained objects we must once more mention the chlorophyll bodies in the plant cell, which are of varying shapes. They lie in the protoplasm and give the green colour to the plant (fig. 5). We have already mentioned two kinds of vacuoles that are met



FIG. 5.—Diagram of Cells with vacuoles and with chlorophyll bodies in the peripherally-disposed protoplasm. (After Sachs.)

with—viz. the occasional watery vacuole occurring in some portion of the protoplasm and the permanent one as seen in plant cells. There is a third kind, termed ‘pulsating or contractile vacuoles,’ which disappear and reappear, *e.g.* amongst the Infusoria (see fig. 1, *a*, p. 36). We have likewise food elements or the remains of undigested food, as in the Amœba (Plate, *a*) and white blood cell, and likewise digestive products. Starch, fat, glycogen, pigment, aleurone grains (in germinating seeds),

crystals, &c., all may appear at one time or another in cell protoplasm, and even parasitic cells may occur, *e.g.* algæ in the cells of the Infusoria.

Let us now consider the structural and other peculiarities of the nucleus, which is so intimately bound up with the life of the cell. Let us first of all consider the nucleus as it occurs in the plant cell. The progress made in the knowledge of its microscopic structure has been due to the elaboration of various fixing and staining methods. Brown is regarded as the discoverer of the nucleus in plants in 1831, and the name of Cytoblast was at one time applied to it. In the higher plants we find it uniformly present in active cells, *i.e.* in those capable of growth and multiplication. It has likewise been demonstrated in lower plant forms, with the possible exception of the bacteria and yeasts, but to this we will refer later on. The nucleus is always found surrounded by protoplasm. In the higher plants down to the mosses there is generally only one nucleus, but more than one may be present, as in certain algæ, and amongst fungi several frequently occur. It may be of various shapes—spherical, ellipsoidal, or with an irregular contour. As regards its chemical constitution there are, so far as we know, two formative groups of substances—the *Proteid* and the *Nuclein* bodies. The *nucleins* appear to be closely associated with the functional peculiarities of the nucleus. The nuclein group is usually divided into Nucleins, Paranucleins, and Plastin. The nucleins are distinguished from the genuine proteids by containing phosphorus, and they are not, or are very slowly, acted upon by digestive ferments, *e.g.* an acid pepsin solution. The genuine nucleins can be split

up by dilute alkalis into proteid and nucleic acid; the latter is a substance rich in phosphorus. From the nucleic acid itself further products can be obtained of the nature of basic bodies and known as the *Nuclein Bases*—*e.g.* Xanthin, Hypoxanthin, Adenin, and Guanin. These bases are rich in nitrogen, and are contained in the nucleic acid in organic combination. The nucleins are most numerous in organs rich in nuclei, *e.g.* in embryonic tissues, and they are present in the most different animal and vegetable tissues. I may cite the formula given for nucleic acid— $C_{40}H_{56}N_{14}P_4O_{28}$. The paranucleins need not detain us here; they are mainly artificially and synthetically prepared bodies, which on treatment with dilute acids yield no nuclein bases, but only phosphoric acid and proteid. *Plastin*, obtained from the *Æhalium septicum*, is, like the genuine nucleins, resistant to the action of digestive ferments. In the nucleus itself is generally to be found proteid matter, nuclein, and plastin, which are distributed between the nucleus and its contained body, the nucleolus. In a general survey of this character it will hardly be necessary to study in detail the differentiation and distribution of substance, as formulated by some observers. In a resting nucleus, *i.e.* one not in process of division, we note a differentiation in structure (fig. 6). We have, *e.g.*, the more refractile nucleolus. There is also an apparent *framework* in the shape of a network—the so-called *Linin*—staining less markedly, and *granules*, which stain deeply the so-called *Chromatin* or *Nuclein*. There is also to be distinguished a nuclear *ground substance*, *i.e.* what remains of the nucleus, after abstracting the framework—viz., the linin and chromatin.

To put it in other words, we have the fluid ground substance of the nucleus, which may have a finely granular appearance, the other nuclear constituents being contained in this. And of these we distinguish an achromatic nuclear substance, consisting of a fine framework, not stainable by ordinary nuclear stains, and a *chromatic* nuclear substance which is stainable, and consists of small granules contained in the strands of the achromatic sub-

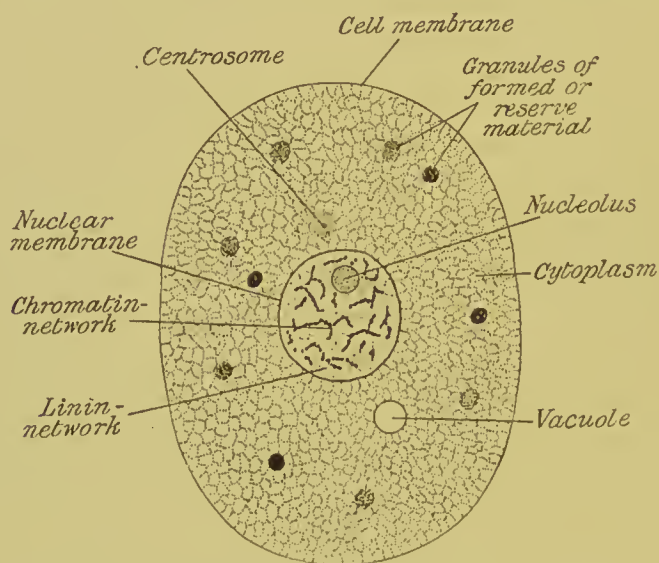


FIG. 6.—Diagram of the structure of a typical Cell.
(Modified from Wilson.)

stance. Also, a homogeneous granule occurs in the nucleus of some cells—the nucleolus—which is probably closely related to the chromatic substance. One generally further distinguishes in the nucleus evidence of an enveloping membrane, and it may contain heterogeneous bodies from time to time. The chromatin granules appear to be the chief seat of the nucleins. As regards the finer structure of the nuclear framework, we have three points of view.

In the first instance, we have Bütschli's theory as to its foamy structure—the chromatin granules being situate at the meshes of the network; secondly, the Flemming theory that the framework consists of a series of threads, with the granules at the points of intersection; and, thirdly, the Altmann theory, which regards its structure as essentially a granular one. Differentiations have not been proved in the ground substance or in the nucleolus. The important part played by the nucleus in cell reproduction will be considered when we come to deal with that subject. The knowledge of its physiology is still in an elementary stage. The nucleus, however, is necessary for the normal development of the cell. Pieces of cell protoplasm separated from the nucleus die, and the nucleus without protoplasm is incapable of self-existence. The nucleus and the protoplasm are both therefore intimately connected, and each is incapable of independent existence; they mutually and essentially influence each other. The nucleus has also been regarded as the supreme agent in the process of cell reproduction. We mentioned that in the vegetable world the nucleus had been demonstrated in all forms, with the exception of the yeasts and bacteria. Some observers, however, state that they have found nuclei in yeast cells. Have the bacteria a genuine cell nucleus? Some observers, such as Migula and Fischer, consider that they show no genuine nuclear structures. Babes considers that the nucleus is represented in the bacterial cells by small granules. According to Bütschli, the whole body of the bacterium surrounded by its cell membrane represents a nucleus or nuclear substance, the protoplasm being only sparsely represented at the

periphery of the cell, or being entirely absent. The bacterial cell would therefore consist of an outer membrane, a peripheral layer, and a central substance; and in the smaller forms only of a membrane and central substance. The central substance stains deeper than the surface layer. And it is true that the stains best adapted for demonstrating bacteria are the nuclear stains. The red-staining granules found in the central substance would then correspond to the Chromosomes of the higher plants and animals.

The general rule in vegetable and animal cells is that there is one nucleus in the cell, and in the nucleus the *chromatin* or *nuclein* is the most characteristic substance. In animal cells we find also the presence of the nucleus, *e.g.* in lymph corpuscles, corneal cells, epithelial cells, &c. It will, however, hardly be necessary to give a detailed description of the nucleus as found in the cells of various animal tissues. We have in our description touched upon the essentials of the nuclei of vegetable cells. Nor need we dwell at this moment upon the nucleus as a possible vehicle for the transmission of hereditary qualities. The essential relation of a third factor to the cell—the Central Body or Centrosome—will be referred to in connection with cell reproduction.

One of the most marked phenomena of organised bodies is their capacity to absorb large quantities of water and of materials dissolved in it; so that in some instances the greater percentage of their substance consists of water. Despite this absorption, however, the protoplasm preserves its feature of insolubility. There are great differences in the form and size of cells. There are cells which are

constantly changing their shape—*e.g.* the naked amœboid cells. The great majority have a constant form, of which the simplest is the spherical. In tissues, as the result of pressure, we obtain various forms, such as the polyhedric. Others may exhibit prolongations of a permanent character—*e.g.* in the brain ganglion cells. Many may have an elongated shape, as in muscle; and some have definite processes for the purpose of locomotion, &c., such as cilia and flagella. The majority of cells are microscopic objects: thus bacterial cells are measured in $\frac{1}{1000}$ parts of a millimetre, and very few cells reach a diameter of a few millimetres.

Having thus discussed in broad outline what is known of the physical, chemical, and morphological properties of protoplasm and of the cell, we may now enter upon the consideration of the salient *functions* of the cell as they present themselves to our notice. We will begin with a consideration of the nature of foods and their assimilation in plants and animals. Organic substance is constantly undergoing phases of composition and decomposition. These life processes centre themselves in the metabolism of proteid matter within the cell. There is a constant building up and breaking down of the proteid molecule. The living cell requires assistance from the outer world both in the form of Matter and in the form of Energy. The materials which supply these two factors may, in the broadest sense of the word, be termed Foods. This raw material is worked up for the requirements of Life in the Laboratory of the Cell. In the course of their passage into and out of the cell, the foodstuffs undergo a series of phases of progressive and regressive metamorphosis, characterised

by various phases of molecular change. The simpler becomes the more complex, passing from a stable to an unstable molecular condition, and the complex passes once more to the simpler condition, from the unstable to the stable phase. In these processes there is no loss of matter; the matter taken up is returned to the environment of the cell, and enters once more into the general circulation of matter of which it is an indestructible part. And the energy likewise is conserved—it is restored in some one phase or other. The food, as a source of material or energy, may be presented to the living plant in one of three conditions—the Gaseous, the Liquid, or the Solid form. There are twelve elements or forms of inorganic matter which enter into the composition of organised substance: these are carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus, chlorine, potassium, sodium, calcium, magnesium, and iron. *Carbon* is presented to the cell in the form of carbonic acid gas. Whilst a small portion of the carbonic acid is free in the air, or dissolved in water, the greater part is present in the soil, where it is united with bases, such as lime and magnesia. The cell may therefore have the carbon presented to it as carbonic acid, in a free or combined condition, and in whatever state carbonic acid may enter the cell, it leaves it again in the same form of carbonic acid. Water and ammonia are the great storehouses in Nature for *Hydrogen*, which mainly enters the cell in these compounds for the formation of the organic substance, to leave again as water and ammonia, or in the form of bodies which ultimately yield these compounds. *Oxygen* may be presented to the cell, free as in the air, or in the numerous

compounds found in Nature which contain oxygen, such, for example, as the molecular groups, carbonic acid and water. A plant is able to absorb carbonic acid and to discharge oxygen; the animal is able to absorb oxygen and to yield carbonic acid. These processes are, however, essentially different, and will be referred to later on. *Nitrogen*, whilst existing free in the air, enters the cell in its compounds—viz. in ammonia and its oxidative derivatives, such as nitrous and nitric acids, notably so in the case of the plants. The plant builds nitrogen up into proteid compounds, and in this form it reaches the animal body; the animal, therefore, depends on the plant for a supply of ready-formed proteid material. The nitrogen leaves the body in the form of compounds, which on their decomposition yield ammonia. *Sulphur* reaches the plant in the form of sulphates, is built up into proteid molecules; and in this form is passed on to the animal, the body of which it leaves in the form of sulphuric acid. *Phosphorus* reaches the plant in a combined form—viz. as phosphates. It enters into the composition of lecithins and nucleins, which are so important for cell life, and mainly reaches the animal as such, leaving its body as phosphates. *Chlorine* exists in Nature in the form of salts, and leaves the body in the form of salts; and so do similarly potassium, sodium, calcium, and magnesium. They do not, however, take part in the formation of organic compounds. *Iron* is mainly found in Nature in the form of its oxidation compounds, in which it acts as a carrier of oxygen to the cell. Its inorganic compounds are absorbed by the plant. It is an important constituent in the human body of hæmoglobin, the blood-colouring

matter, whilst in the plant it is essential for the formation of chlorophyll. In certain plants and animals copper seems to replace iron.

It will thus be seen that the physiological chemistries of the animal and vegetable kingdoms are interdependent and closely related. Bunge makes the following useful division of foods into three categories :—(1) those foods which equally serve as a source of energy and as compensative substances for lost constituents of the body—*e.g.* proteids and fats ; (2) those which only serve as a source of energy—*e.g.* oxygen and the carbohydrates ; (3) those which only serve as compensatory substances for lost constituents, but not as sources of energy—*e.g.* water and the inorganic salts. These are the main ways in which the essential elements are presented to the cell for the purpose of its vital processes. The preliminary modification brought about in the foods thus presented represents *Digestion*, whereby the essential constituents are changed into an assimilable form. The incorporation of these essential elements as derived from the foods into cell substance or into protoplasm is a constructive process termed *Assimilation*. The breaking down of cell substance is a destructive process by which simpler bodies result, and is termed *Dissimilation*. If we bracket the two phases of assimilation and dissimilation together, we get the circle of processes to which the collective name of *Metabolism* has been applied. Of these the constructive processes have been grouped under the word *Anabolic* ; the destructive under the word *Katabolic*. Cellular metabolism, therefore, consists of anabolic and katabolic processes. Whatever the ultimate product may be, Life

derives its proximate material elements from the inorganic world. Let us now shortly consider what these vital products are; this will help us greatly in our consideration of vital phenomena. We must at the outset bear in mind that the elements exist in the cell in the form of a specific and very unstable molecular structure, that the chemical methods of analysis gravely modify this molecular constitution, and that the products of analyses do not represent the conditions existing in the cell. We are able to trace the several processes more at the periphery than at the centre. The proteid bodies are essential to the life of the cell, which centres itself in their peculiar metabolism. The proteid bodies contain the five elements carbon (C), hydrogen (H), nitrogen (N), oxygen (O), and sulphur (S), and may be termed the nitrogenous constituents of the cell. They cannot be dispensed with; life cannot go on without them. They have a very complicated structure, as will be seen by the formula suggested, for example, for hæmoglobin, $C_{600}H_{960}N_{154}FeS_3O_{179}$. The size of the proteid molecules is therefore very great, and they do not diffuse through parchment membranes; they are usually colloids, not crystalloids. Hæmoglobin is an example of a crystallisable proteid. The solid proteids of the food are rendered soluble or converted into an assimilable or diffusible form by the action of certain cell ferments. This is essentially a process of hydration, and results in the formation of soluble bodies—the peptones. The proteids differ in their solubility in water, and in this way certain broad differentiations may be set up. We have, for example, the *Albumins*, which are freely soluble, such as egg albumin. We have the

Globulins, only soluble in water in the presence of unsaturated solutions of neutral salts; on saturation a precipitation occurs—*e.g.* plant and serum globulins. And we have the *Vitellins*, only soluble in neutral salt solutions, but *not* precipitated by saturation—*e.g.* the aleurone bodies of the seeds. Whilst the above forms of proteids may occur in the body in a free condition, a large number are chemically bound—*e.g.* *hæmoglobin* is a combination of albumin and iron. The *nucleins* are a combination of albumin and nucleic acid. The *nucleo-albumins* are complex compounds of albumin and nuclein. In *casein* we have the combination of a nucleo-albumin with lime, and we may have, as in *mucin*, the combination of albumin with a carbohydrate. There are other bodies which in many respects behave like the albumins, and are therefore termed *Albuminoids*—*e.g.* elastin and keratin. Finally, we have proteid derivatives in the cell, such as the ferments or enzymes—*e.g.* pepsin. As well as such progressive we have likewise regressive processes going on, and the setting free of various metabolic products. Amongst these we have heterogeneous bodies, such as urea, uric acid, and kreatin, and nuclein bases such as xanthin, &c. We have likewise the release of non-nitrogenous products such as carbonic acid, lactic acid, and various carbohydrates such as sugar, glycogen, &c.

In the *Carbohydrates* we have bodies of simpler constitution than the proteids. They are very important constituents in plant life. They contain no nitrogen, and are built up out of the three elements, carbon, hydrogen, and oxygen, the hydrogen and oxygen being in the proportions to form water $(H_2O)_n$ —*e.g.* $C_6H_{12}O_6$. Of

these we have three main groups:—(1) the *Mono-saccharides*, of which $C_6H_{12}O_6$ is a typical formula—*e.g.* grape sugar. They are readily decomposed by the action of ferments into carbonic acid and alcohol— $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$; (2) the *Disaccharides*, such as are represented by the formula $C_{12}H_{22}O_{11}$ —*i.e.* two molecules of a monosaccharide from which a molecule of water has been abstracted, *e.g.* cane and milk sugars. One of their main properties is their capacity for inversion into monosaccharides through the action of acids or bacteria, thus becoming converted into a fermentable condition; (3) the *Polysaccharides*, in which we have the combination of several monosaccharide molecules, each with the loss of a molecule of water—*i.e.* a multiple of $C_6H_{10}O_5$, as starch, glycogen, and cellulose. We may have various decomposition products of the saccharides, such as carbonic, lactic, and butyric acids.

In the *Fats* we have compounds of an acid with an alcohol, with the loss of water. They are built up of carbon, hydrogen, and oxygen. Their basis as regards an alcohol is glycerin; this is combined with a fatty acid, *e.g.* stearic acid in some fats, and in oils with oleic acid. The neutral fats can therefore be decomposed into glycerin and fatty acids, and the free acids unite with alkali to form soaps. The fats are soluble in and readily extracted by ether.

Amongst the *Inorganic* constituents of living substance we have water, which, in a combined or free state, may form a large percentage of the organism, *e.g.* 50 to 59 per cent. We have likewise the various inorganic salts to which reference has been made, and finally the

gases, such as oxygen and carbonic acid. In all cells the essential phenomena rest in the metabolism of the proteid bodies. What are these metabolic processes of which the cell is the seat? It may be broadly stated that the plant is capable of building up its substance from inorganic material. The animal cannot do this, but requires previously formed organic material, for the supply of which it is dependent on the plant. We shall have to consider the manipulation of gaseous, liquid, and solid elements, and will discuss briefly not merely *digestion* and *assimilation*, but include at the same time in our survey the *respiratory* process which is common to all cells. In the case of naked protoplasm, the food can be taken by the cell in a solid form as in the Amœba, with the direct absorption into its interior of the green alga cell. Oxygen, likewise, is taken up in this way. This is, however, the exception, the majority of cells possessing some definite membrane. Whilst in such instances a gaseous exchange may take place through the cell walls, and salts or other soluble substances may pass dissolved in water into the cell, the solid food must first be converted *outside* the cell into a soluble and diffusible condition; and this is a process which the cell undertakes on its own behalf. One of the most remarkable features presented by a living cell is the *selective* capacity it shows for food—it accepts some forms of matter that are presented to it and rejects others. The Amœba seeks out and selects the alga cell, the Vampyrella attaches itself to its prey, the Spirogyra and the intestinal epithelial cells take up fat globules and reject other similarly sized particles that may be present. The cell in its nutritive processes may be ministering

entirely to its own needs—as in the case of the free-living vegetable cell, the Bacterium, or the free-living animal cell, the Amœba—or it may take up food not only for itself, but on behalf of the general community of cells in which it finds itself. The food may by means of the *sap* be distributed to the various tissues of the plant, or by means of the *blood* be carried to the remotest tissues of the animal body. In such instances we look for and find specialised cells, tissues, and organs which minister to the complex requirements of the higher organisms. The faculty of manipulating the solid food outside the cell rests in some kind of soluble ferment or *enzyme*. The enzyme is prepared in the cell, and can be secreted under the stimulus of the presence of solid food, and it performs its work outside the cell. These enzymes are of great interest, and a few words may be devoted to their consideration. At this moment we are considering them mainly as the means of converting indiffusible into diffusible substances. These ferments are produced both by animal and vegetable cells, and it is usual to speak of *organised* and *unorganised* ferments. The organised are those in which the ferment action goes on inside the living cell, and in which the living cell is apparently necessary to the process. The unorganised ferments are those whose action is able to take place without the direct intervention of the living cell that produced them. To *these* the terms are applied of soluble ferments or enzymes. We know more of the effects than of the nature of these enzymes. Their isolation and examination in a pure form have not yet been attained, and the nature and action of the organised or living ferments

present an equally difficult field of research, as they are so intimately connected with the activity of the living cell itself. The yeast cell has been spoken of as a living ferment, the gaseous alcoholic fermentation of sugar appearing to take place only in the presence of the living yeast cell, though some suppose that its fermentative action is due to a body of the nature of an enzyme. But as it is a classical example, it will serve to illustrate the conception which led to the distinguishing of organised ferments. If we take a pure culture of vegetable cells, *e.g.* the *Bacillus megaterium*, and add it to a nutrient medium, which has been rendered solid by the addition of gelatin, we find that the organism in the course of its growth completely liquefies the medium, and converts the solid gelatin into a fluid peptone. If we render the fluid free from cells or inhibit their action by chloroform, and add a few drops of the liquefied gelatin soil to fresh gelatin, we find that this is in its turn liquefied, in virtue of a soluble body which has been produced and secreted by the bacterial cells into the surrounding medium, and which is capable of exerting its action apart from the living cell which produced it. Here we have a typical example of an unorganised soluble ferment or enzyme. The ferment in the course of the process remains unchanged, and the action of each ferment is a specific one directed towards given groups of substances. As I said, whilst we can follow their effects, we know little or nothing of the exact nature of the ferments. They are the seat of a peculiar form of energy, which produces the discharge of the potential energy of chemical substances, and causes its transformation into Actual or Kinetic Energy. The less

stable chemical substance is changed into more stable modifications, with stronger affinities and with less potential energy than the original substance. The process is, at any rate, one which becomes visible to us outside the cell (an 'exo-' not an 'endo-cellular' process). It has become difficult to find satisfaction in the terms 'organised' and 'unorganised' ferments, meaning thereby processes of a similar character and running their course in virtue of a substance or form of energy produced by the cell, and acting by *contact*, only that in the one case it is free and in the other it is anchored to the protoplasm.

LECTURE IV.

The Ferments or Enzymes—Pro-ferments — Various Kinds of Ferments and their Action—Nutrition and Assimilation—Excretion—Osmosis—Cell Growth—Protoplasmic Movement.

In the last lecture we were occupied with a further consideration of the cell as the Unit of Life in some of its closer aspects. We found that vital phenomena rested on the activity of cells, whether we considered these phenomena in their simple or complex manifestations. We proceeded to touch upon what was known of the more intimate structure of the cell, and stated that present-day inquiry regarded protoplasm with its nucleus as the integral and essential constituents of this vital unit. The traces of structure revealed in the cell and the nature of the same next claimed our attention, and furnished an introduction to our consideration of the functions of the cell itself. We touched on the meagre knowledge we possess of the chemical and physical properties of protoplasm, and we referred to the twelve chemical elements which are found at the basis of all organised matter. We next considered the various forms in which food is supplied to the cell, and found that this supply may take place either in a gaseous, a liquid, or a solid condition. We noted that the food has usually to be

converted into an assimilable form, and that this is brought about by the direct intervention of the cell or of certain immediate derivatives of its living substance, the most remarkable and characteristic of these being the soluble ferments or enzymes, which passed out of the cell and were able, so to speak, to act as the deputed agents of the protoplasm itself. Whilst we are aware of their effects, the nature of these active agents or enzymes is obscure, and many of our deductions regarding them are of a hypothetical nature. We are now in a position to examine more closely the action of these soluble ferments, and the nature of the effects they produce. I will mainly refer to those which yield us some salient and general conceptions of their mode of action.

As regards their chemical action, the ferments may be divided into two groups—(1) the hydrolytic, and (2) the oxidative ferments. The hydrolytic act by splitting up a more complex into a simpler molecule, with the addition to the molecule of the elements of water, viz. by hydration. We see this process in connection with the carbohydrates and their transformation by the action of diastatic ferments, such, for example, as occurs on starch, resulting in the production of dextrin and maltose. And as regards proteid bodies, we have the action upon them of the pepsins, whereby albumoses and peptones are produced. And, further, we have the fat-splitting ferments, whereby the fats are decomposed into fatty acids and glycerin. As regards the *oxidative* ferments, we have many which occur in plants and bring about a transference of oxygen, *e.g.* the oxidation of ethyl alcohol by the acetic acid ferment.

The enzymes which will concern us at present are not those which are anchored to the cell protoplasm, but those which are secreted by it. It does not seem to be the case that these particular enzymes exist as such in the cell, *i.e.* in a pre-formed or active condition, but rather in the condition of a pro-ferment or 'Zymogen,' as it is usually termed.

In accordance with this theory the zymogens are in themselves inactive, but become active ferments or enzymes on contact with certain chemical substances, notably dilute acids. We find these ferments in ALL phases of cellular Life, whether it be in the plant or the animal, and whether we consider the simple vegetable cell or the specialised animal cell. There is, however, this to be noted, that whilst in the simple unicellular organism we may find a number of enzymes occurring as the product of *one* cell, we find them in the higher organisms produced more individually by the specialised cells of special organs. The ferments have been found amongst the bacteria, the yeasts, the moulds, and in the juices and tissues of the higher plants. In the lower animals they have been found in the insects, as well as amongst fish and amphibia. Amongst the higher animals they are to be notably met with in special organs, and likewise in the tissues. They further fulfil an important function in *young* developing organisms as in germinating seeds, and also in the case of animals (*e.g.* in the embryonic stages).

To refer to them now in more detail, we may commence with the *Proteolytic ferments*, which transform proteid substances into simpler compounds. There are three main groups of these proteolytic ferments. *Pepsin* is a typical example of the first group, as secreted by the

gastric or stomach cells of vertebrate animals. In virtue of its action proteids are converted into albumoses and peptones, and this generally occurs in a faintly *acid* solution of the ferment. We have a second group of these proteolytic enzymes, which act best in neutral or slightly alkaline solutions and which produce deeper changes in the proteid molecule. A typical example is *trypsin*, as secreted by the pancreas. The end products of its digestive action are simpler bodies than is the case in pepsin digestion, *e.g.* amido-acids and basic substances. The third group of these ferments are the *Coagulating* or *Curdling enzymes*. Of these the *rennet* or milk-curdling ferment is a typical example. All these ferments perform important functions in the life processes of animal and vegetable cells. They may have special seats of formation—pepsin in the gastric cells and trypsin in the pancreas cells. The proteolytic ferments are widely distributed; amongst plants we find them in the simple bacterial cell and in the cells of insectivorous plants. Their action can be demonstrated in fluid cultures of micro-organisms, and they can be extracted from the cells that produce them by the aid of glycerin. The pepsin exists in cells in an elementary or rather potential stage as *pepsinogen*. The pepsinogen is quickly converted into the active pepsin by means of carbonic and other acids. It has been demonstrated in almost all vertebrate animals and is found likewise in a number of invertebrates. In all cases diffusible bodies or *peptones* are the result of its action. In the course of the *tryptic* digestion we have to note, besides the appearance of peptones, the occurrence of other bodies, such as leucin and tyrosin. As already

stated, the trypsin acts, at any rate in the test tube, most energetically in weakly alkaline solutions. The trypsin, like pepsin, is found in the cells in a *zymogen* stage.

We find sundry powerful proteolytic ferments amongst various species of plants. We may mention *papain*, the energetic proteolytic ferment found in the *Carica papaya*, as well as similar ferments in the fig-sap and in the pineapple, viz. *bromelin*, and a number of others might be mentioned. We have already referred to the proteolytic ferments of the bacteria, moulds, and insectivorous plants; the organism of ringworm produces a remarkably active ferment. All these ferments are capable of passing out of and acting independently of the cell to which they owe their origin. And no doubt there are a number still to be demonstrated, especially if tritulating and pressure methods be applied to obtain the active juices of cells. We have already mentioned the rennet ferment of the calf's stomach, which in the presence of lime salts curdles milk and probably splits the milk casein into two proteids—viz. whey proteid and the paracasein. We have similar milk-curdling ferments amongst the plants, *e.g.* in the *Carica papaya*, as well as in the artichoke, thistle, &c. The bacteria likewise are producers of milk-curdling ferments. Another important group of ferments are the *Saccharifying enzymes*. They are most widely distributed amongst plants and animals. They fulfil an important function, which consists of transforming the more complex carbohydrates, such as starches, into the simpler sugars, which can then be used for the vital processes of the cell. The *specific* action of the proteolytic enzymes on the proteids is paralleled by

the specific action of the saccharifying enzymes on the carbohydrates. Of these saccharifying enzymes there are certain types to be borne in mind. We have, in the first instance, the *Diastases*. Under the term 'diastase' we understand an enzyme which can, in virtue of its hydrolytic action, produce out of starch dextrins and the sugar maltose. The diastases are found in many plant and animal tissues; they are to be met with in malt, in the cryptogams, in the pancreas, and in the liver. They are probably, like the proteolytic enzymes, of a proteid nature and derivation. The dextrins which result from their action (erythro- and achroo-dextrins) are non-crystallisable products. As a further indication of their wide distribution, we may mention some of their seats—germinating barley, germinating seeds such as oats, maize, rice, &c., plant saps containing starchy matter, algæ, fungi, yeast infusions, &c. They are cell secretions, and are closely connected with the starch-dissolving properties of the plant. In the higher plants the diastase appears to exist in the cells in a potential form or zymogen. We have several examples of an interesting symbiotic action of ferments, as, for example, in the Koji yeast, which forms the Japanese rice-wine—*Saké*. It contains a mould (*Aspergillus orizæ*) which yields a diastatic ferment, and acts along with an alcohol-forming *Saccharomyces*, or yeast. The same conditions are found in the Tonkin yeast, the *Amylomyces Rouxii*, a mould producing the diastatic, and a yeast the alcoholic fermentation, and there is likewise a symbiotic action in the ginger-beer plant. The diastatic enzymes are likewise widely found amongst the bacteria. In the animal

economy such ferments have an important function, converting the unabsorbable starch and the reserve glycogen into soluble sugars. They are found especially in the saliva, in the intestinal juice, and in the liver and pancreas. The saliva diastase produces dextrins and maltose, and similarly the pancreatic ferment. The liver contains a starch-like carbohydrate—glycogen—which is formed from the grape sugar of the blood, and is easily reconverted into sugar. This process is likewise of a fermentative nature, and it may be said that these diastatic ferments are widely distributed in animal tissues and organs. There are certain diastase-like ferments which are of considerable interest—viz. the cell wall or cellulose-dissolving enzymes. The endosperms of many plants contain reserve cellulose or cell-wall forming substances, which become dissolved in the process of germination, *e.g.* in the barley grain. They have been termed *Cytases*, but require further investigation. It may be that wood-destroying parasites act in virtue of such enzymes. They possibly convert the cellulose into soluble sugary bodies, and in Nature the bacteria undoubtedly play an important part in the redistribution of the decaying cellulose elements of plants. Amongst animals an active cytase has been found amongst the snails. We have further ferments which attack the disaccharides, *e.g.* *glucose*, which splits maltose into two molecules of glucose. It is widely met with amongst plants and animals, and generally accompanies the diastatic ferments. Its most important seat is in malt extract, and almost all yeast cells contain it. In the saccharification of starch by animal enzymes, we note that besides maltose grape sugar also is formed.

There is likewise *invertase*, which converts cane sugar into glucose and fructose. Invertase is present in yeast cells, in moulds, and in some bacteria, and is to be met with in the intestinal juices and in the organs of animals. We have further the *glucoside* ferments. We have seen that the yeast contains two enzymes—maltase and invertase. These have a *specific* action, *the one attacking maltose and the other cane sugar*. E. Fischer has advanced the hypothesis that the specific action is due not only to structural differences, but to differences in the molecular configuration of the substances attacked. If we take a glucoside containing α and β glucosides, we find that the yeast ferment only attacks the α glucoside. The β glucoside can, however, be attacked and decomposed by emulsin. We have, therefore, for these complicated derivatives of the sugar two different sets of ferments, each with a specific action. Emulsin is found in almonds, and splits the glucoside amygdalin into grape sugar, benzaldehyde, and prussic acid. Emulsin is also found in other plants and amongst the bacteria, and it appears to occur in some animals.

The enzymes are frequently only formed if the decomposition of the substance in question is of physiological significance for the given organism. Thus, as regards a bacterium, if sugar is present it will attack this and not amygdalin. It is an interesting fact that mustard oil does not exist pre-formed in the plant seeds, but is first formed on contact with water. This is due to the action of a ferment *myrosin*, which splits the glucoside, potassic myronate, into grape sugar, potassic sulphate, and mustard oil. Most of the *Cruciferæ* contain myrosin.

The myrosin and the glucoside are formed in *different* cells. One sees, therefore, how the presence of *water* enables them to come together.

We have likewise a series of *Fat*-splitting ferments—the *Lipases*. They split neutral fats into glycerin and free fatty acids—*e.g.* the pancreatic enzyme. The free fatty acid becomes emulsified by the sodic carbonate of the intestine. Such enzymes are to be met with in animal organs, *e.g.* the kidneys, and in blood serum. They are met with in fishes and insects, as well as in germinating seeds and in moulds. We have *lactic acid* fermentations, due to the action of microbes, and amongst these organisms it is a widely distributed property. Lactic acid is also formed in dying muscle. The alcoholic ferments need not detain us at present; they form a special chapter in the biology of the yeasts. As an example of the *Oxidases*, we may take *salicylase*, which oxidises salicyl aldehyde to salicylic acid. These oxidising ferments are present in plants and animals, and demand much further inquiry.

We have now proceeded so far in our consideration of the functions of the cell that we have obtained a general outline of one of the primary conditions of Life, viz. *nutrition*. We have seen that all organic substance is built up out of a few ultimate elements which exist in the inorganic world. These are rarely presented to the cell in the form of the simple elements, but almost entirely in the form of their combination. Whilst the plant cell is able to assimilate these elements in the form of inorganic compounds, the animal cell requires that they should be presented in a more elaborated form, viz. in the shape of already-formed organic substances. The animal is

dependent on the plant world for the preliminary synthesis of these indispensable bodies. Proteid nutriment is absolutely necessary for the life of the animal cell; and whilst all cells show a remarkable selective power as regards their food, the form in which such food is presented to them is not an indifferent matter. It may have first to undergo a process of *digestion*, or, in other words, a conversion into an assimilable condition. This usually means the conversion of the solid food into a liquid or diffusible form, whereby it is enabled to penetrate into the cell. We found that according to the nature of the food the cell is able to depute agents which perform this necessary work. These agents are not only of a varied, but likewise of a specific, character, and may attack either proteids, as, for example, pepsin; carbohydrates, as, for example, diastase; or fats, as, for example, lipase. There is *no* complicated phase of chemical decomposition which the cell is not able to set in action for its own purpose, and in this respect the ferments or enzymes play a leading, if not the leading, part. And we have seen that these ferments are as ubiquitous as life itself.

On the other hand, *ready*-formed *liquid* nutriment may be directly taken up, as is largely the case in the plant cell, whilst gaseous substances can be directly absorbed by the protoplasm—*e.g.* oxygen and carbonic acid.

The assimilated food is subjected to the metabolic processes of the cell, which are centred in the metabolism of proteid bodies, whether of a progressive or a retrogressive character. We have, however, in doing this but touched the preparatory stages of assimilation. The assimilative process in itself varies according to the nature

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and quality of the cell in question. In the plant we find, as stated, the formation of organic from inorganic matter. The assimilated carbon of the carbonic acid undergoes a remarkable metamorphosis; the carbonic acid is split up, the oxygen passes out of the cell, and the carbon disappears. What becomes of it? The carbon thus obtained is built up into the starch molecule of the plant cell. The starch molecule likewise contains hydrogen and oxygen, and these the plant can assimilate from the elements of *water*. The metabolic process here, therefore, presents itself to us as a synthesis of carbonic acid and water to form the starch molecule, and starch appears in the plant cell. The starch, however, is only a *reserve* substance; it can be used as required in the building up of structure. We have seen how readily it is converted by the plant diastases into soluble sugars and transported to the tissues where it is required, there to undergo various modifications and yielding up to the cell the essential elements, carbon, hydrogen, and oxygen. We have already noted that the proteids are nitrogenous bodies, containing in their molecules nitrogen united with carbon, hydrogen, oxygen, and sulphur. How do the plant cells obtain the necessary nitrogen and sulphur? They cannot do so from the trinity of atoms in the carbohydrates, *i.e.* the starches or sugars; they must find some *other* source. The nitrates and sulphates absorbed by the root cells of the plant furnish to them these essential proteid constituents. The means whereby carbon, hydrogen, nitrogen, oxygen, and sulphur are converted by the cell into a proteid molecule are, however, hidden from us. In the *animal* cell the carbon, hydrogen, nitrogen, oxygen,

and sulphur necessary for its proteid molecule do not undergo synthesis from inorganic matter, but are directly furnished in the proteid foods obtained from plants. These proteids are simply converted into assimilable peptones. In the animal body we find the peptones on the one side of the intestinal cells, but not on the other, *i.e.* in the blood—they have disappeared. The progressive metabolism which they undergo would, therefore, appear to rest in the cells of the intestinal wall, *i.e.* the process of their conversion into proteid molecules, which can be seized upon by the cells generally of the body and transferred from the blood to their substance, finally to reappear and to be excreted in the course of retrogressive metabolism as urea, uric acid, &c. As we have seen in the case of the epithelial cells, fat is taken up as such and can act as a reserve material. The starchy food can be saccharified and the grape sugar formed converted into a reserve of glycogen in the animal cells. In the retrogressive metabolism of the proteids nitrogenous and non-nitrogenous compounds result. Amongst the nitrogenous we have urea, uric acid, &c., and amongst the non-nitrogenous carbohydrates, fats, carbonic acid, &c. There is everywhere a tendency in these dissimilative processes to return to *stable* substances similar to those which originally entered the organism or cell, and thus to furnish fresh material for the processes of life in a gaseous, a liquid, or a solid form. The useless material is thrown off in the form of *excretory* substances which pass from the organism to the outside world. We may, however, have a *utilisation* of substances for building up intercellular or skeletal framework; for example, the

formation and thickening of the cell membrane in plants, the formation of cartilage and bone in animals, as well as protective coverings as seen in the shells of numerous animals. The simplest form of *excretion* is represented by the VACUOLE of the single-celled organism which reaches the periphery of the cell and empties itself, as in the *Amœba*. There is also the *external* secretion of mucin or viscous substances which in a simple animal serve to anchor it to a surface or to lubricate the surface of a membrane in compound tissues or organs. There are further to be noted the various pigments, perfumes, &c. Carbonic acid is an excretory product from *every* cell in the course of proteid metabolism, and oxygen from the green-plant cells in the course of the metabolism of carbonic acid. Water and dissolved substances may be excreted by stomata in plants or by glandular tissues in animals. We have already mentioned the nature of the nitrogenous excreta; the non-nitrogenous are generally discharged as carbonic acid and water. In cells which take solid food, solid excretions may occur. Some of the cell products are exquisitely poisonous, as, *e.g.*, the toxalbumins of the pathogenic bacteria, such as the diphtheria bacillus. In conclusion, the end products of all proteid metabolism are those with which the cell started—viz. carbonic acid, water, and nitrogenous salts.

We may now consider more closely the cellular metabolism along with the intimately connected function of respiration. And in the first instance we will mainly consider the cells of the green plant; and in this connection the relation of water to the plant cell is of first importance. It is to be noted that the healthy protoplasm is always

in contact with water. The water enters the cells by a process of Osmosis, *i.e.* the movements of fluids which take place through a homogeneous, permeable membrane. If the fluids on each side of the membrane are of different density, they tend to pass through the membrane in both directions until there is a mixture of *equal* density on each side. This diffusion or osmotic process varies with the concentration of the solution. The familiar example is water and syrup; if these are separated by a membrane, a stream of water passes to the syrup through the membrane and a stream of syrup to the water. At first the flow of water is greater, *i.e.* towards the syrup, until equilibrium becomes established. Many other substances can set up osmotic currents. In a young plant cell with a cell membrane, osmotic substances with an affinity for water are contained in its protoplasm. The water passes into the cell, which in consequence becomes distended. The cell eventually contains more water than can be absorbed by the protoplasm. The surplus fluid collects in drops, and runs together to form a watery vacuole. This vacuole may eventually occupy the greater portion of the cell interior, being surrounded by a peripheral layer of protoplasm (see fig. 5, p. 65). The cell, however, does not behave like a dead parchment membrane. We have to take into account the vital activity of the protoplasm, which intervenes between the cell wall and the vacuole, and which, whilst allowing the passage of water, may oppose the passage inwards of various osmotic substances dissolved in it, and in a similar way prevent their passage outwards. Thus a 10 per cent. solution of sodic chloride abstracts water from the cell, and the protoplasm shrinks from the

cell wall. The salt solution likewise passes through the cell wall, but not through the protoplasm, which retreats before it. If we replace the sodic-chloride solution by water the cell vacuole re-forms and its original condition is regained; there is a regulative action of the protoplasm on the process. Such osmotic currents continually pass from cell to cell. The cells likewise produce osmotic substances, so that they are continually attracting and absorbing water, and in consequence become distended; the cell is then said to be turgid, and the condition one of turgor or turgescence. This turgidity may be so great as to force droplets of water out of the cell, and the condition promotes an interchange of water. For purposes of nutrition *dilute* solutions of salts can pass into the cell, and likewise gases, whilst similar substances may escape from the cell. There is, further, *evaporation* from the surface of cells in a plant tissue into the intercellular spaces, and this will aid in producing currents from cell to cell. These phenomena of interchange, resting upon variations in the osmotic power of a cell or cells, will ultimately influence a wide series of cells. This circulation of water does not merely promote nutrition with dissolved salts; it likewise favours the transference of oxygen to the cell and the removal of carbonic acid in the course of respiration. In addition to these diffusion currents from cell to cell, we have in plants the passage upwards of water from the soil, by means of the woody fibre, to the leaves and aerial portions. This ascending sap starts in the delicate root hairs; the turgescence of the cells of the root hairs exercises a hydrostatic pressure, and water is forced into the vessels and upwards. The root pressure

thus exercised promotes an upward circulation of water. Further, we have an evaporation of water from the surface of plants, known as *Transpiration*, the exits being furnished by small openings or stomata, guarded by cells which, by opening or closing the apertures, can regulate the transpiration process, and may likewise regulate the circulation of the watery sap.

We have, therefore, noted as the main factors in this circulation (1) the *root pressure*, (2) *evaporation* or transpiration, and (3) *osmosis*, as in the cells of the leaves, under the controlling action of the living protoplasm. This general circulation is not merely a means of circulating water with dissolved nutrient substances amongst the cells; it likewise aids in cell aëration, gases diffusing through the moist cell membranes, notably carbonic acid and oxygen. The absorption of oxygen and the discharge of carbonic acid are the beginning and end of the vital intracellular process of *respiration*. The process is an absolute necessity for the metabolism of all cells, which by the oxidative splitting up of organic molecules obtain their necessary vital energy; if they cannot obtain oxygen they die. This thirst of the cell for oxygen must be satisfied, and the process is carried on in the simple cell as well as in the highest organism in which, by means of the lungs, the oxygen is hurried through the channel of the blood to the various cells of the body. The process is accompanied by the discharge of carbonic acid and the production of heat. In a germinating seed the temperature may rise as much as 15° C. above that of its environment.

An important distinction must, however, be borne in mind. Whilst the vegetable cell absorbs oxygen and secretes carbonic acid, it is likewise capable of carrying out the reverse process—viz. of absorbing carbonic acid and secreting oxygen. We must keep these two processes distinct in our minds. The absorption of oxygen and the release of carbonic acid constitute a *respiratory* process; the absorption of carbonic acid and the release of oxygen an *assimilative* process; the one is respiration, the other is assimilation. The respiratory process is common to animal and vegetable cells, but the assimilative process, as mentioned, is confined to vegetable cells, and amongst these it is not general, but is limited to cells which contain the green colouring matter, chlorophyll: in other words, to chlorophyll-containing plant cells. Oxygen absorption or respiration is an oxidative decomposition process; carbonic acid absorption or assimilation is a synthetic process—a weaving of carbon into the organic substance of the plant cell, while the released oxygen leaves the plant. The first evidence of this assimilation in the cell is the appearance of starch. The process requires light, or, in other words, heat, which is supplied by the sun to release the oxygen from the carbonic acid and water. In respiration heat is set free; in assimilation it is bound. If we chloroform a plant, the assimilative chlorophyll function is stopped. In the process of assimilation of carbonic acid in the plant cell we have the *first synthesis of organic out of inorganic matter*, a bridge thrown over and connecting the animate with the inanimate world. It is no wonder, therefore, that this transformation has its seat in a specialised apparatus or

mechanism in the green plant—viz. the chlorophyll apparatus.

Broadly speaking, the root cells absorb nitrogen in the form of ammonia and other compounds, *e.g.* the nitrites and nitrates, and likewise absorb the constituents of the ash, whilst gases enter from the soil or by the leaves from the air. The absorption of carbonic acid takes place in the chlorophyll apparatus. The chlorophyll is generally associated with definite protoplasmic bodies, which have been termed *Plastids*, and appear to possess a reticulated structure, which contains the soluble pigment in its meshes (fig. 5, p. 65). This pigment gives to the whole body a green colour, and the bodies have hence been termed chloroplasts or chloroplastids, the chlorophyll being kept in solution by some oil-like substance. The chloroplasts are usually round or oval bodies, situated in the general protoplasm of the cell. The chlorophyll requires a certain temperature for its production, and likewise the presence of oxygen and iron; if there is no *iron* present the cells lose their green appearance. Its function, as already indicated, is the synthetic formation of some kind of carbohydrate from the elements of carbonic acid and water, but we do not know what the stages in the process are. A sugar of some kind would appear to be the first product, and there is likewise soon the appearance of starch. Some suppose that *hexose* ($C_6H_{12}O_6$) is the first carbohydrate formed, whilst others consider the primary product to be cane sugar. The starch formed by the chloroplasts can be converted into sugar by the diastases and transported to the cells that require it. As a result of metabolic changes in the

starch, fatty oils are produced in the plants. We have already touched on the formation of the plant proteid molecule ; and the experimental proof has been furnished by cultivating plants in solutions containing various kinds of salts.

The carbohydrates and proteids serve as food for animals, and in their cells they are oxidised ; their potential is converted into actual energy, whereby work is carried on and heat is produced. To put it in another form, in the plant processes of reduction and synthesis occur ; in the animal those of oxidation and analysis. The *fundamental* processes of life, however, both in the vegetable and in the animal cell, are the *same*, with this exception : that the plant has developed a special function, which resides in its chlorophyll apparatus. The non-chlorophyll vegetable cell is in a similar situation to the animal cell, neither possessing a chlorophyll apparatus. We thus find the large class of saprophytic and parasitic bacteria without chlorophyll dependent on proteid material for their nutriment. These bacterial cells perform in this way a most useful and indispensable function, as by their action a large quantity of organic *débris* in Nature is cleared away by their action, which, if it accumulated, would render all life impossible.

We have touched on the transport of starch to cells in the diffusible form of sugar, which on reaching the cell requiring it can be re-stored as starch ; and a similar process occurs in animal cells. We have already mentioned the action of ferments in this process. The difficult question is the exact part taken by the protoplasm in cell metabolism. It may be that the organised substance is

formed out of the molecules of the protoplasm itself, or that it is built up out of plastic substances, such as peptonised proteids, carbohydrates, fats, &c., which are absorbed and separated in an organised condition. For example, does the cell membrane arise as an organised substance directly out of the protoplasm, or is it formed out of plastic carbohydrates as an insoluble modification, viz. cellulose? Is it a transformation or a separation product? Is the process an indirect or a direct one? Possibly it is the former, an indirect process. We have internal and external plasma products, and it may be useful in the discussion of metabolism to gather together what is mainly known of these, even at the risk of repetition. Amongst the internal products we have vacuoles containing fluid and dissolved substances, as met with in plant cells and in the lower animals. Likewise fats, glycogen, starch, and mucin, which are temporarily stored for subsequent use, nor must we forget the pigments. We have permanent products, such as the skeletal framework of plants and animals, either arranged internally or externally. Amongst external plasma products we have (1) membranes, (2) cuticular tissues, (3) intercellular substance, respective examples being cellulose, plant and animal cuticles, mucin, chondrin, and gluten. Tannin is a general plant-cell product, along with by-products such as resins and alkaloids.

The nitrogenous plastic material in plants is probably converted into some amido-acid, *e.g.* asparagin. It should be mentioned that the deposit of starch is generally brought about by the agency of small protoplasmic corpuscles, the leucoplasts, which are situated near the

nucleus. They absorb sugar and secrete starch in their substance. Amongst the fungi the carbohydrate reserve may be stored as glycogen. Sugar is likewise stored in the cane and the beet. In the aleurone grains of the seed we have proteid reserves stored, and in cereal grasses viscous substances such as the glutins, and likewise a storage of glucosides; their food value is partly due to the sugar they contain. And, finally, we have fats and oils as contained in numerous seeds. The enzymes which attack these reserves may be secreted in the reserve material cells or in cells close to the reserve cells, and may be carried to the reserve cells by the connecting strands of protoplasm.

We have the evidence of rudimentary digestive glands in plants, *e.g.* in seeds there is a special organ, the scutellum, separating the embryo from the endosperm. The side in contact with the endosperm has cylindrical cells, which contain two enzymes, which are discharged into the endosperm to effect digestion. This may be termed a diastase of secretion and is localised, whilst the diastase of translocation is generally distributed.

At the moment of the secretory act, the cell protoplasm enlarges and becomes granular. The granules are passed out in solution into the sap, and the protoplasm resumes its normal appearance. The granules probably represent a zymogen, or potential ferment.

To put some of our considerations in more general phraseology, the plant for the discharge of its work requires energy, and the sources of this in the green plant are the rays of the sun, which enter the plant in an actual or kinetic form, and are stored in the form of

potential energy. Each cell is a seat for the liberation of potential, in the form of kinetic, energy. The storing of material means a supply of potential energy. The destructive metabolism of the cell, therefore, becomes a source of kinetic energy, and this depends on processes of chemical decomposition. In respiration we have a utilisation of stored cell energy, *i.e.* a change of potential into the kinetic form.

Where free oxygen is not available for the cell, it may be taken from substances such as the sugars, as occurs with anaërobic bacteria.

As a result of the metabolic processes of the cell, we have the phenomenon of growth—a permanent increase in substance and in bulk, and a permanent change in form. The increase in substance is an anabolic process, the manufacture of framework a katabolic process. Growth depends mainly on the following conditions: (1) a supply of nutritive material; (2) a supply of water to set up a certain hydrostatic pressure in the cell; (3) formation of osmotic substances to induce the entrance of water; (4) a certain temperature; and (5) a supply of oxygen for the liberation of energy. We have thus in the cell water absorption, with turgidity and vacuolisation, and a cell wall formation or thickening. We have already indicated the influence of environment on these several processes. In health the reaction between the organism and its surroundings is a perfect one and the organism is in a condition of tone, and the external factor which brings this about is called a tonic influence. The external factors which mainly vary are light, heat, and moisture. The cell must stand in a proper relation to these in order to be healthy, and

their influence, according to the degree, will be a normal or an abnormal one. As regards temperature, there are maximum, minimum, and optimum limits set for each species of cell, within a range usually extending from 0° to 50° C.

The environment of the cell calls forth certain modifications in structure. Thus the degree of surrounding moisture affects the cell. In aquatic plants we find an absence of woody tissue, and a development of the same in desert plants. Parasitic plants have their structure modified in virtue of their mode of life, and in the fungi degenerations in organisation result. As regards light, its influence on the formation of chlorophyll at once occurs to us, none being formed in its absence. And many examples might be given of the effect of environment in aiding, in degrading, or in modifying function, the result being a normal, an increased, or a lessened metabolism, and leading, it may be, to death. We have spoken of the condition of health in an organism. This condition we cannot represent by a straight line, but rather by a curve, starting at the birth of the cell, climbing to its zenith and the moment of reproduction, to fall away on the other side to the ultimate extinction of its vital processes.

We have now sketched in broad outline the chemical, morphological, and physiological properties of protoplasm, and have proceeded some way in the consideration of function. We have dealt with the nature of foods, and with digestion and assimilation, along with the nature of excretory and secretory metabolic cell products. We have seen how energy is supplied and how it is applied in the discharge of vital function as well as the essential nature

of the respiratory process. Structure lies at the basis of function, and with change of function structure may undergo various modifications, as, *e.g.*, in the chlorophyll and muscle cells. In these respects the effect of environment is of a varied and wide-reaching character. At the same time the essential phenomena of Life rest on a common physical basis, and are to be traced to, and find their origin in, a vital unit, the *Cell*, which unit focusses all essential vital expressions—viz. nutrition, growth, reproduction, motion, and sensation.

Life in its normal aspects is a continual reaction of vital functions to external stimuli. This sensitiveness on the part of protoplasm is its characteristic vital feature. We may, therefore, now conveniently consider some of the results of stimulation on the cell.

One of the most obvious effects of stimulation is some form of motion on the part of the cell protoplasm, and to this we will devote a few words. These movements may be of the protoplasm itself or they may occur within it. We must be careful, in the first instance, to exclude such passive movements which are common to all particles in a fluid. Thus, the red corpuscles in the blood move in virtue of the pumping action of the heart on the fluid blood, and likewise particles of matter may be carried about in the streaming movements of protoplasm itself. The Brownian molecular movements are those which occur when organic or inorganic particles of matter are suspended in a fluid. All these, however, are of a passive character. The active movements of protoplasm as exhibited under the influence of stimuli are different, and will form the opening subject of the next lecture.

LECTURE V.

Protoplasmic Irritability and Movements—Response to Stimul —
Influence of Temperature on Cell Activity—Phototaxis—
Chemotaxis—Galvanotaxis—Cell Reproduction and Division.

Having considered broadly nutrition and growth in the last lecture, we had reached the consideration of a most important property of cell protoplasm, viz. its irritability, its capacity for response to various external agents or stimuli, this response being characterised usually by some form of visible motion. We must in the first instance be careful to exclude certain movements which are not of an active, but of a passive character. Thus, granules may be carried along in a movement of the living substance, just as chips are carried with a stream of water; and the blood cells may be passively hurried along in the circulation of the blood stream as a result of the pumping action of the heart. Further, any particles of matter suspended in a fluid may exhibit restless and dancing movements, commonly termed 'Brownian motion.' All these, however, are *passive* movements. Those with which we are here concerned are *active* movements on the part of living matter. These active movements may be of a varying character. Thus we may have: 1, movements of the protoplasm itself; 2, motions due to appendages of the protoplasm, such as cilia and flagella; and 3, movements

such as are seen in the pulsating vacuoles of certain cells. Let us first consider the protoplasmic movements. These may occur in naked protoplasmic cells, such as are to be found amongst the unicellular organisms. We have already referred to the active movements of the *Amœba* by means of the pseudopodia or extensions which its protoplasm is able to throw out and whereby it is able to effect changes of place. These movements may be directed towards some special object of prey — for example, an alga cell. Thus the *Vampyrella* seeks out given algæ (*Spirogyra*) for the purpose of nutrition.

Such active movements are likewise well marked in the wandering cells of the human body known as phagocytes. These cells direct themselves to bacteria which have entered the system, absorb them into their body substance, and digest them in the same way as the *Amœba* absorbs and digests alga cells (fig. 11, p. 249). In inflammatory processes in the body tissues the white blood cells pass through the walls of the minute blood vessels or capillaries. And amongst the slime fungi or Myxomycetes we have a pseudopodial motion which enables these organisms to move against the stream in a current of water. In the protoplasm of these organisms flowing movements likewise occur, as is evidenced by the movement of granules which are carried along with the moving protoplasm. All the movements that have just been mentioned occur in naked cells, *i.e.* in cells destitute of a confining wall or cell membrane.

There are besides these certain protoplasmic movements which take place *within* cells possessing cell membranes. These movements are especially to be met with in the cells of plants. The movements are usually

described as being of a twofold nature: (1) movements of rotation, and (2) movements of circulation. The movements of rotation are seen, for example, in the cells of the *Characeæ*, in which there is a large central sap vacuole surrounded by protoplasm. The protoplasm differentiates itself into two layers, of which the outer layer is at rest, and the inner exhibits a rotary motion, so that it passes round the entire inner surface of the cell. The *circulatory* motion can be seen in the *Tradescantia* cells. The phenomenon is similar to that which is seen in the protoplasm of the *Myxomycetes* or slime fungi. One notices a streaming of granules contained in the protoplasm, and starch and chlorophyll granules may thus be carried along with the current. These streams of granules pass along the bridges of protoplasm as well as along the peripheral portions of the cell protoplasm. The appearance is a striking one, but an explanation of this phenomenon has not yet been satisfactorily given.

We come now to the *second* form of motion as exhibited by cilia and flagella. These bodies are very fine continuations of the protoplasm, projecting out in a whip-like fashion from the cell. They are permanent and not temporary cell appendages or outshoots, as in the case of the pseudopodia of the *Amœba*, and consist of a contractile thread of protoplasm derived from the cell protoplasm itself. We find them amongst the zoospores of the fungi and algæ, in virtue of which the cells are endowed with the power of independent locomotion. If the cell has a membrane the cilia or flagella pass out through fine pores in the membrane. We may have them occurring in

large numbers on the entire surface of a cell, and these minute whips are known as *cilia*, or they may be fewer in number and longer, and these are known as *flagella*. The cilia are usually either at one or other end of the cell, and the cell may thus either be pulled or propelled forwards. As a result of the motion the cell, being somewhat specifically heavier than water, is kept floating in the watery medium, or is propelled in given directions. The Infusoria have a large number of cilia, and hence have been called Ciliata. The cilia are very small and numerous—it has been estimated that a paramœcium cell possesses about 2,500—and exhibit a co-ordinated movement, and appear to be under the influence of stimuli arising from the protoplasm itself. The cilia by setting up currents can drive food into the neighbourhood of an organism and in the fixed cells of the tissues, as in the ciliated epithelium of the respiratory passage, foreign bodies can be transported outwards (fig. 7,

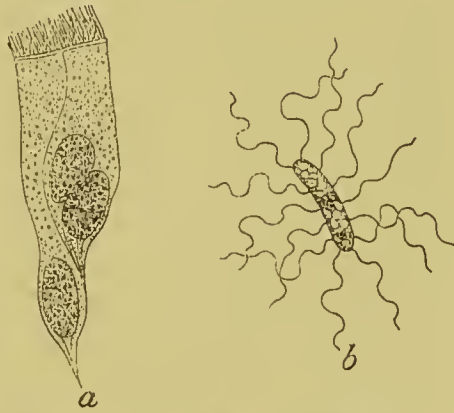


FIG. 7.—*a*, Ciliated Epithelium; *b*, Typhoid Bacillus with Flagella.

a). Amongst such minute cells as the bacteria, which are measured in $\frac{1}{1000}$ parts of a millimetre, movements are frequently brought about by the aid of flagella. This can be seen very well in the case of the typhoid bacillus, which has a number of flagella ranged around its body, and which are much longer than the organism itself (fig. 7, *b*). In

some protozoa, such as the trypanosomes, flagella are well developed (fig. 13, p. 261).

We have finally the contractile vacuoles of unicellular organisms, *e.g.* in the *Amœba* and *Infusoria*. They empty and refill at regular intervals, disappearing and reappearing in the process. They are probably elementary cell organs of importance for respiration and excretion. One cannot refrain from mentioning the ingenious means by which certain *Rhizopods* can change their level in water, rising by the development of a bubble of carbonic acid within their protoplasm and sinking by its release. The *Arcella* is a unicellular organism with a shell of concavo-convex form. In the middle of the concave side is an opening through which pseudopodia can be thrust. If the *Arcella* falls on its back the pseudopodia have nothing to which they can grip. Gas bubbles then arise in the protoplasm near one margin, and that side becomes specifically lighter; it rises, and the animal gets on to the other sharp margin. The pseudopodia can now grip the substratum, and the animal turns round and the bubbles disappear. The *Arcellæ* are able in this way to place themselves in any suitable position. If we consider more closely the amœboid motion we find that it is a contraction phenomenon—a phase of expansion and a phase of contraction. The expansion phase is a stretching out of the pseudopodia; the contraction phase is a flowing back of the protoplasm and a return to the spherical form. We have, therefore, a centrifugal and a centripetal motion, with the spherical protoplasm stage as the central point.

In the muscular movement of higher organisms we have likewise the phenomena of contraction. Even in very

simple organisms we may find this development of contractile tissue—*e.g.* the *Vorticella* has a muscular thread attached spirally to the inner wall of an elastic sheath, forming a stalk which serves to fix the cell body, and the stalk may contract into a spiral and so draw with it the organism. The complicated structure or the histology of striped muscle in man need not detain us here. In its contraction each segment becomes shorter and thicker ; in expansion the reverse occurs. Amœboid, ciliary, and muscular movements are, therefore, contraction phenomena with a phase of alternating contraction and expansion, through a change in place of portions of the living substance.

We now come to a closer examination of stimuli and their action. An organism does not find itself constantly under the same conditions. The external conditions are continually varying, and call forth responses on the part of the cell. If these are adequate we find in the cell a healthy or normal performance of its functions. The effect which environment has had on structure and form we may see in the long processes of developmental change which have occurred since the Primeval Cells commenced their existence ; and at every moment in its life history the cell is being stimulated, and is emitting responses more or less adequate to such stimuli. These responses may not attract our observation if they are phases of modified intracellular phenomena or if they are the slow result of a long-continued external influence. On the other hand, they may be immediately noted by us, as in the case of artificially applied stimuli. The visible effect of some such applied stimulus usually manifests itself to our perception in some form of motion of the cell itself, or of parts, or of

tissues. Amongst the stimuli which may act we find heat, light, and electrical, chemical, and mechanical stimuli. The response will vary and depend on the nature of the specific object affected. Thus a stimulus to the eye calls forth the sensation of light, one applied to a gland produces secretion, to a muscle contraction. This power of stimulation is common to *all* cells. A small local stimulus may further produce a wide effect through the transmission of the stimulus, as in the case of the human nerve. One can illustrate the effects of such stimuli very well in the case of the thermic stimuli. Temperature is one of the most important conditions for the activity of living matter. Life generally is conditioned by certain limits of temperature, *i.e.* there is a maximum and a minimum temperature beyond which life becomes impossible and death ensues. At one time the temperature of the earth rendered life impossible, and a time may come when the conditions will once more be impossible for vital activity. Between these two extremes of cold and heat there exists for every cell a degree of warmth at which its activities reach their highest normal development. This temperature is termed the optimum temperature of the cell, and varies for different cells, approaching in one instance towards the minimum and in another instance towards the maximum temperature at which life is possible. The degree of resistance of cells to changes in temperature conditions varies greatly. The maximum of *heat* for animal and vegetable cells usually ranges round 40° C. We may illustrate the range of temperature very well on the remarkably adaptive vegetable bacterial cells. These cells have an extraordinary range of temperature,

extending from 0° C. to 70° C. There are bacterial organisms which can grow and develop even at freezing point, and there are organisms which thrive at 70° C., a temperature at which most forms of protoplasm become lifeless. Between these extremes we have numerous variations in the possible range of temperature. We find organisms which flourish best at 15° to 20° C., and organisms which flourish best at temperatures approaching the blood heat—viz. about 37° C. The thermophilic or heat-loving bacteria have an optimum temperature of 55° C. We have, therefore, optimum temperatures which may range themselves round 15° C., 37° C., and 55° C., with all stages of transition between these temperatures as regards individual species of cells. It may be generally stated that cells are more sensitive to changes in temperature passing above the optimum than to those passing below the optimum temperature. The bacterial cell is able to withstand enormous plunges below the freezing point—*e.g.*, an exposure to the temperatures of liquid air and hydrogen—without losing its recuperative vitality. On the other hand, as a general rule, an elevation of temperature to and above 60° C. quickly destroys the life of the cell, except in such instances as the thermophilic bacteria. So varied are the temperature conditions under which cellular life is possible that the optimum temperature for one organism may be the thermal death point for another. Increased temperature up to a certain limit produces increased activity. In the case of the yeast cell we have, *e.g.*, an increase of carbonic-acid production with an increase of temperature up to 30° to 35° C., when the cell metabolism becomes so active that the fermenting

liquid foams. The *Amœba* responds at first to increased temperatures with livelier movements, and the white blood cell carefully heated begins to stretch out pseudopodia, which movement increases as the optimum temperature is approached. The *Amœba*, whilst reacting to increased temperature by livelier movement, as the temperature rises above the optimum point lessens its activity, and at 35° C. contracts to a spherical form. In the same way protoplasmic movements in plant cells increase, but eventually at 45° C. the protoplasm assumes a spheroidal form. Similarly ciliary and muscular movements may first be increased and then finally inhibited. If we decrease the temperature below the optimum we get similar effects at 0° C.; the yeast ceases to ferment and the *Amœba* is motionless and spherical. We see therefore that, stopping short of death, a paralysis of function occurs under the influence of temperatures which shade away either above the maximum or below the optimum temperature for the cell in question. If we may so express it, we have in the case of the cell a heat rigor or a cold rigor, which, if the stimulus is increased or is prolonged, becomes the *rigor mortis*. Heat stimuli may also exert a directive force or *Thermotaxis*; thus the plasmodium of *Myxomycetes* shows to heat a positive thermotaxis, it is attracted; and the *Amœba* exhibits a negative thermotaxis, it is repelled. Enough has been said to indicate the importance of the thermic stimuli for the processes of life and to illustrate their effect on the simple Unit of Life—the Cell.

Let us now consider the response of living matter to another form of stimulus—viz. *Light* or photic stimuli. And in this instance it is the chemical and not the

thermic action of light that we have to consider. We know how great is the influence of light on vitality, that the life of the plant is due to sunlight, and that therefore all life is dependent for its existence upon the influence of the light rays of the sun. When a plant is placed in darkness and kept under such conditions it loses the property of reacting to its surroundings—its power of stimulation becomes lost, and amongst other effects we notice that its leaves cease to grow. The normal condition in which it exists under the influence of light is known as *Phototonus*, and the condition is one of tone—the tonic influence being light. A Rhizopod when suddenly exposed to the light withdraws all its pseudopodia and contracts to the spherical form. On the other hand, the *Bacterium photometricum* moves in the presence of light, and ceases its movement in the dark. In a plant the growing parts turn to the light and exhibit a positive phototaxis, as it has been termed. The spores of chlorophyll-containing algæ in moderate diffuse light collect at the side of the drop turned towards the light, but if the light becomes more intense they migrate away from it. In the case of the bacteria the action of direct light is generally injurious, and their successful culture requires that they should be kept in the dark. The tubercle bacillus, for example, dies very quickly when exposed to the direct rays of the sun, and diffuse light has a slower but a similarly injurious action. In light the skin of the frog acquires a clearer colour, the black pigment cells of the skin under the action of the light having contracted into small dark globules. We have to differentiate between general and between irregular and one-sided

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stimuli; it is the latter which produce movements in a given direction. The spores of green algæ in a vessel collect on the side next the light; the plasmodia of the Myxomycetes, on the other hand, collect on the shaded side. The reaction to light is therefore varied in individual and collective instances. In a chlorophyll-containing alga, light has a directing influence on its chlorophyll apparatus; in subdued light this arranges itself at right angles to the rays; in intense light it comes to lie more in the direct line of the rays of light. If the light is intense and of long continuance the chlorophyll apparatus may shrink. In the words of Stahl, 'The chlorophyll granules protect themselves from a too intense light by twisting, by an emigration to another part of the cell, or by a change of form. In weak light the largest surface is turned to the source of light, and as much light as possible is taken up. With stronger illumination the opposite behaviour occurs, inasmuch as a smaller surface is turned to the light.' Enough has been said to illustrate the effect of light as a stimulus on cellular function, and how every cell seeks to establish a normal condition for itself and to place itself in a state of phototonus as regards light. Where the influence of light is more one-sided, we have movements in a given direction. We may have in the readjustment which the cell finds it necessary to make for itself a visible or traceable movement which may be of a positive or a negative character. Just as the cell may exhibit positive or negative reaction in the case of heat stimuli, so it may exhibit in the case of light stimuli a positive or a negative *phototaxis*—that is to say, attraction or repulsion.

We now come to the consideration of the important subject of *Chemical Stimuli*. Changes in the chemical constitution of the surrounding medium will produce changes in the cell. The changed chemical conditions act as stimuli in various ways. If the change is a gradual one, the cell may adapt itself to the changed surroundings. An addition to the food may stimulate the metabolic processes of a cell; we have the difference between a poor and a rich soil and its influence on the activity of organisms. In the case of the *Amœba*, a solution of salt will stop its movements and cause it to assume a spherical form. Acids and alkalis may produce a rapid increase of ciliary movements and likewise contraction in muscles. The *Amœba* in the absence of oxygen contracts, and when oxygen is added it once more expands. The addition of salts may in the case of phosphorescent organisms induce a bright phosphorescence. We have likewise the action of narcotising agents. Chloroform depresses all cell metabolism; in the presence of chloroform the *Mimosa* ceases to exhibit its spontaneous movements, the yeast cell ceases to ferment, the cilia of a cell cease to move, a muscle ceases to contract, and in a nerve the motor and sensory functions are paralysed. Chloroform does not, therefore, act merely on the nervous system, it acts as a narcotic on every kind of protoplasm.

As with the other forms of stimuli described, chemical stimuli may result in movements which may likewise be termed in this special instance a positive and a negative *chemotaxis*—the cell is attracted towards or is driven away from the chemical substance in question. As regards gases, oxygen exerts the most marked attractive action

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upon cells. This can be illustrated on the plasmodium of the *Myxomycetes*. If a beaker be filled with water which has been boiled to drive out the contained oxygen and covered with a layer of oil to protect it from the air, and then a piece of filter-paper is placed in the beaker so that one half of the filter-paper is above and the other half below the oil layer, *i.e.* one half is in contact with an oxygen-free atmosphere and the other half is in contact with an oxygen-containing atmosphere, and if on the paper a plasmodium is deposited, one finds that the plasmodium retracts itself from the oxygen-free water, climbs up the paper, and settles at the part which is freely exposed to the air.

It has likewise been shown that many bacteria are amongst the finest reagents we possess for detecting the presence of traces of oxygen. If to a fluid containing bacteria alga cells are added, the alga cells are soon surrounded by a thick cluster of bacteria, which are attracted by the oxygen given off in the process of chlorophyll assimilation by the alga cells. If such a cell moves and shakes off the swarm of organisms, they still follow it and once more cluster thickly round it. As regards fluids, we have again the instance of the slime fungi. If these organisms are spread out on damp filter-paper, as soon as the paper begins to dry they draw back to the parts which remain moist. And similarly as regards chemical substances, the plasmodium is attracted to a tan decoction, for example, and is repelled by a solution of salt. In this way, by positive and negative chemotaxis, the cell can approach suitable and avoid unsuitable substances. If a 0.01 per cent. solution of malic acid be placed in a fine capillary

tube, it attracts the fertilising threads of the fern, and they enter the capillary tube. Commencing at a minimal value lying at about 0.001 per cent., the attractive action increases with the increasing concentration of the malic-acid solution up to a given point, when the optimum or maximum effect of the stimulus occurs. By further increases in concentration of the malic acid the attractive power decreases, and there finally comes a moment when the positive changes into a negative chemotaxis. How small the amount necessary to produce a positive effect will be seen when it is stated that it can be induced by a 35-millionth part of a milligramme of malic acid.

On the other hand, other species of cells may be driven away by the malic acid, *i.e.* a negative chemotaxis occurs. A 1.0 per cent. solution of meat extract will attract many bacteria into a capillary tube. We have in the human body the white blood cells, which react similarly with a positive or a negative chemotaxis, *e.g.* a capillary tube filled with an extract from pus organisms, and placed in a frog's lymph sac, becomes filled with corpuscles. In the subcutaneous tissues such substances derived from the bacterial cells attract leucocytes. The leucocytes are therefore capable of responding to chemical stimuli proceeding from bacterial substances, and in this they follow the general laws of positive and negative chemotaxis. Chemotaxis therefore plays an important part in life processes, whether of a physiological or a pathological character. A chemical substance may be indifferent, or it may be positive or negative, as regards the stimuli it exerts on a living object or cell.

We now pass to the consideration of another kind of stimulus, generally known as *Barotaxis*. All mechanical influences, such as pressure, shock, squeezing, &c., act as stimuli on protoplasm—they produce a change in the pressure conditions. If a cell be exposed to shaking or if it be squeezed, the protoplasmic movements are seen to cease. Similarly the *Amœba* draws in its pseudopodia and assumes the spherical form. A touch applied to a pseudopodium will cause it to shorten. We have also in simple touch or contact a positive barotaxis, whereby a living object keeps attached to a given substratum, as, *e.g.*, a plasmodium to its substratum; it flows along it and does not rise. Also in clinging plants we see the same positive phenomenon.

We have further the *geotactic* phenomena in plants, whereby they place their radial axis in a given direction relative to the middle point of the earth, and thus exhibit definite movement—the roots show a positive and the stems a negative geotactic property as regards the earth.

Finally, *Galvanotaxis* must be considered. When a galvanic current passes through the protoplasm of a cell it acts as a stimulus. In the *Tradescantia* it stops granular movements, and the protoplasm clumps. A slight galvanic current temporarily stops amœboid movement, a stronger stream produces the spherical form in the amœba, and a long-continued current can even break up and disintegrate small unicellular organisms. In the case of the *Paramecium*, when the current is closed the organisms swarm to the kathode pole, and when the current is opened they scatter through the fluid. Some infusoria are attracted, on the other hand, to the anode. If ciliated infusoria

and flagellata are present they fly in different directions : the flagellata to the anode and the ciliata to the kathode pole. In this way a mixture of anodic and kathodic organisms can be separated by the galvanic current ; we may have, therefore, a positive and a negative galvano-taxis.

As a result of any form of stimulation a cell may show evidence of exhaustion, with consequent changes in its substance. A stimulus may produce, first an exaltation, and eventually a depression of functional activity ; and if the stimulus is excessive death may result, or if it be normal a condition of tone prevail.

We have now illustrated the basal phenomena of Life, and shown how they have their seat in the Cell, which may therefore be termed the Unit of Life, viz. as regards the cardinal functions of nutrition, growth, motion and sensation. In conclusion, a few words may be devoted to a vital phenomenon which represents a crisis in the cell life to which all its energies tend, and which it reaches in the climacteric period of reproduction, all cells being derived from pre-existing cells. The cell does not merely seek its own self-preservation ; it likewise provides for the conservation of life. And this it does by producing new cells of its own kind. The originating process of the new individual is known as *Reproduction*, and we may sufficiently illustrate our thesis of the Cell as the Unit of Life by a consideration of the reproductive process in the unicellular and multicellular plant organisms. The life of all organisms, however complicated, derives its origin from a cell. The process may be described roughly as one of cell division. It is only comparatively recently that the

significance of the nucleus has been appreciated in the process. The nucleus in the process of cell reproduction does not disappear to be reformed in the new cell; it takes an active share in the process of cell division; it likewise divides, and, in fact, appears to initiate the process. The new nucleus, like the cell, is formed from previously existing nuclear substance. Our definition of the cell therefore holds good, the structural unit of life being represented as a speck of protoplasm containing a formed body—the nucleus. The division of the protoplasm is a simple phenomenon; the division of the nucleus is a complicated process. Every cell reaches a stage in its growth or development when it undergoes a process of multiplication by division. In some unicellular organisms in which there is no distinct differentiation into protoplasmic and nuclear substance, as in the bacteria, the process is a simple one of transverse fission; the spherical organism divides, the rod-shaped organism becomes cut into two, and similarly the spirillum divides into two new cells. In every case of cell reproduction a cell division is at the bottom of the process. We have likewise a simple form of cell multiplication in the case of the yeasts, viz. a process of budding or germination; a bulging of the cell at one point occurs, which increases in size, and so a daughter cell is formed, and is finally cut off from the mother cell by a process of constriction.

In the *Amœba* we have a unicellular organism with a distinct nucleus. In the process of cell division the nucleus becomes elongated, constricts in the middle, and divides into two new nuclei. The protoplasm then divides or constricts, and eventually ruptures between

the two new nuclei, and the result is two fresh Amœbæ (see fig. 1, p. 36). The above processes may be summed up in the words *direct cell division*. But the majority of cells undergo another process of reproduction, viz. that of *indirect cell division*, and to this we must devote a few words. In this process the visible division of the protoplasm is a simple cell-division process, but as regards the nucleus remarkable and complex phenomena can be distinguished. In this process, known as karyokinesis, the nucleus takes a *leading part*, and there are many analogies in the process as it occurs in plant and animal cells. The one thing common to all the processes of indirect cell division is the transference of nuclear substance and protoplasm substance to the daughter cells. The cardinal features of the process are centred in the nucleus. As we noted in a previous lecture, the nucleus essentially consists of a fine fibrillary network of chromatin or chromatic substance (nucleins), which is embedded in a hyaline or achromatic substance, the whole being surrounded by a differentiation of the protoplasm, known as the nuclear membrane (fig. 6, p. 68). The chromatic substance forms what one may term the nuclear framework; the achromatic substance consists of numerous delicate threads arranged in a spindle shape, known as the nuclear spindle. In the *chromatic nuclear* substance, in the course of division, we can distinguish that in the first instance the chromatic substance arranges itself in threads, which appear loosely rolled up like a ball of thread. The threads divide longitudinally, and in this way *double* threads or fibres are formed. The nuclear membrane disappears, and at opposite poles of the nucleus there

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appear the central bodies or *centrosomes*, each of which is united by a thread-like figure of achromatic substance, now arranged in the spindle form of the nuclear spindle. The double threads group themselves in a bent form at the equator of the achromatic nuclear spindle, with their angles directed to the middle point. The constituents of the double threads are then drawn apart, so that one half of each double thread is drawn to an opposite pole. In this way the threads are withdrawn from the equator of the spindle and recede to the poles. The spindle threads now become indistinct, and the threads curl up at each pole into a ball. The cell develops a ring-like constriction, which divides the cell into two equal parts, each with a newly formed nucleus. The spindle threads disappear, and each nucleus develops a membrane, and assumes once more the resting stage. This process is found in the most varied cells, and consists essentially in the transference of nuclear as well as protoplasmic substances.

This concludes our general survey of the Cell as the structural Unit of Life; and I think we have been able to trace that, however complicated the phenomena of Life may be, they have their origin and derivative seat in the Cell, which forms the centre round which all vital functions are grouped and in which all the fundamental functions of Life itself may be illustrated—viz. nutrition, growth, motion, sensation, and reproduction.

CELLULAR PHYSIOLOGY

A COURSE OF SIX LECTURES
DELIVERED AT THE ROYAL INSTITUTION
APRIL AND MAY 1901

LECTURE I.

Cellular Structure of all Plant and Animal Tissues—Fermentation
—Historical Retrospect—The Alcoholic Fermentation.

In the previous course of lectures the subject dealt with was the Cell as the Unit of Life. An attempt was made to sketch in general outline the activities which are peculiar to living matter, and an account was given of the physical basis on which these activities rest in so far as our present knowledge reaches. We found that living matter, whether it be of plant or of animal origin, exhibits certain fundamental properties, and that these properties or functions are common to all living organisms and are to be met with in the humblest as well as the highest types of life. These cardinal and essential functions are evidenced in the phenomena of growth, of nutrition, of reproduction, of sensation, and of motion.

These form the basis of all living activity. The plant increases in substance—it grows; it absorbs nutriment—it feeds; it forms the embryo of a new plant in the seed—it reproduces its species; it reacts to various external stimuli such as light and heat—it exhibits sensibility, and as a result of some form of stimulus it may exhibit distinct movements either of an external or of an internal

nature. And in the same way we noted identical phenomena in the animal, *i.e.* the same basal vital activities.

If we take a green plant and examine it we readily note that the plant is composed of various parts—it possesses structure. These parts of its anatomy—the *tissues*—respectively perform some function in connection with the life of the plant as a whole. They may, as in the root, serve for the absorption of food elements from the soil; they may, as in the case of the leaf, serve for purposes of respiration and transpiration; or, as in the case of the green parts or chlorophyll apparatus, for the transference of carbon from the carbonic acid of the air into the living substance of the plant. The coarse anatomical structure of the plant is *not*, therefore, simple; it is built up of various parts or tissues. Nor are these parts or tissues of a simple or of a uniform character—as is revealed on a closer examination of them with the aid of the microscope. On magnification it is seen that the component tissues consist of a honeycomb of small chambers or cells. Just as a house is built up of a large number of units—*bricks*—so the plant tissue is composed of numerous units which have been termed ‘cells.’ The structure of all plant tissues is a *cellular* one. What are these cells or units of which it is composed? The essential structural features of the cell appear to us to consist of a ground substance, to which the name ‘protoplasm’ has been applied, along with a contained body, known as the nucleus. The cell, therefore, in the modern acceptance of the word, is a tiny speck of protoplasm within which is a nucleus. This unit, this cell, is in the great majority of plant tissues surrounded by a containing wall or cell-

membrane, although this is not an essential feature in the life of the cell itself. Wherever we search in the plant we find these bricks or structural units going to make up its tissues. All the activities of the plant tissues or of the plant as a whole are based on this cellular structure, *i.e.* on the activities of the individual cells of which they are composed. For example, the phenomena of growth are eminently bound up with an activity and increase of cell substance, and the powers of reproduction rest in the cell, consisting essentially in a process of multiplication by the division of individual cells. Each cell is the product of a previously existing cell. The cell, we may say, is the central point in all plant life. And if we consider *animal* structure we find, likewise, a number of diverse parts or tissues entering into the composition of the various organs. These tissues, examined more closely, although they may perform widely differing functions, show just as in the plant the same organisation out of cells; their structure is likewise a cellular one. This cell or unit, as in the plant, consists essentially of a ground substance, protoplasm, with a contained body, a nucleus. And it is on this cellular basis that the vital functions of the animal rest, its growth and reproduction depend on cell increase, on cell division and multiplication. Each new cell springs from a pre-existing cell, and in this way the organism approaches and reaches the full development of its structures and functions. Everywhere, therefore, we trace the cell and its activities. And if we extend our study to forms of life so minute that their existence is only revealed to us by the microscope, we find a whole world of organisms in which the

individual consists of a single cell. These single free-living cells lead an independent existence, and exhibit all the essential activities of life which are to be noted in the higher plant and animal. They are not one of a number of bricks; they lead a completely free and independent existence as living units. Indeed, these free-living cellular units, as revealed to us by the microscope, far exceed in numbers the ordinary visible forms of life. A teaspoonful of soil may harbour millions of these forms, as in the case of the free-living vegetable cells, known as the Bacteria. And if we turn to the animal world we find a wealth of similar microscopic forms, leading, as in the case of the *Amœba*, an independent life, each unit exhibiting the cardinal functions of growth, nutrition, reproduction, sensation, and motion. The same essential vital phenomena as seen in the highest plant and animal are found to exist in the lowest free-living animal or vegetable cell. The plant and animal, however complex their structure may be, are, as we have noted, composed of congeries or republics of cells, with the same essential properties. You will see, therefore, that there is justification for the intimate connection drawn between cellular and vital activity, and for regarding the cell as the unit of life, whether we regard that life at the beginning or at the full development of its activities, or whether we consider the functions themselves or the structure on which they rest—viz. the cell protoplasm with its nucleus. If, in the highest and most complex organisation—MAN—we trace the functions of each tissue and organ, we find that they have their seat in the cells of which these tissues and organs are composed. The phenomenon of contraction in a muscle has its seat in

the muscle cells, nervous phenomena have their origin in the nerve cells, and glandular secretion is due to cellular activity; whilst in the plant the unique functions of its chlorophyll apparatus are essentially of a cellular origin. However wide the circle of life may be drawn, and however remote the periphery at which we touch it, the cell is the centre to which all observation leads us. The problems of vital activity and their solution lie *there* in the cell and its functions. No wonder, then, if in recent years the study of the cell has been placed in the forefront of investigation. If the main problems of vitality rest there, the end and aim of physiological inquiry must be the *cell*. If in the study of function, whether in the plant or the animal, we are led away from the cell, we are ultimately brought back to it. However far away the goal may still seem, our aims will and must tend to the foundation of a Cellular Physiology.

Though I have just stated the facts in a very brief and bare fashion, it took indeed a long time to develop and to establish the cellular doctrine as regards living matter. It was a long road that led to the discovery that breathing or respiration is a function common to all living matter, and that it is essentially a cellular phenomenon. The cell takes up oxygen and discharges carbonic acid, whether it exists as a simple free-living unit or as a fixed constituent in the complex body of the higher plant or animal. The things that have baffled us so long have been mainly the mechanisms employed in this or in other vital processes—the secondary or subsidiary arrangements which facilitate, but which are distinct from and have really nothing to do with, the essential features of the process itself. These,

of course, occur in the higher and more complex plant and animal organisms. The simple animal organism, such as the *Amœba*, takes up oxygen from and discharges carbonic acid directly into the surrounding medium. The complex animal organism, as in the case of man, pumps air or oxygen to the blood by means of a pair of bellows—the lungs—the oxygen is taken up by the blood cells and is hurried to the tissues, *i.e.* to the tissue cells, which require it for their life processes. A great part of physiological inquiry has consisted in the examination and the explanation, *not* of life but of the mechanism of life, and so far as this mechanism is concerned, adequate and satisfactory explanations have been found in the ordinary laws of Physics. It is when we come to cellular activity that our real difficulties begin as regard the essentially vital problems. It is, therefore, a most valuable biological conception to have reached, even if it ultimately prove to be of but relative finality, that all living matter is structurally cellular and of cellular origin, and that function rests in and is a property of cells, and that what goes to make the cell is protoplasm with its nucleus, or, as one may perhaps better put it, the physical basis of life consists of protoplasmic and nuclear substances, and in these vital activity centres. This is far removed from the old conception, based mainly on botanical study, that the containing wall or membrane was the essential feature of the cell. The modern view is truer to the facts, inasmuch as in the course of cell multiplication, we find that so far as our observation goes there is a transference not only of protoplasmic, but also of nuclear, substance to the daughter cells; whilst the protoplasm severed from the nucleus, or

the nucleus severed from the protoplasm, is each incapable of independent existence. We have thus passed from an analysis of the tissue resulting in a cellular doctrine to an analysis of the cell which consists of protoplasm and nucleus, and this is as far as our present analytical methods permit us to go. We have, it is true, arrived at the definition of a vital unit, but the conception is mainly a structural, a morphological one, and does not deal with the essential phenomena, but with the basis on which they rest. It is, however, an advance to know that protoplasm and nucleus are essential to life, and that such a unit is constituted by a cell which is capable whether in a free or in a fixed condition of manifesting all the essential vital functions.

What is the nature of living protoplasmic and of living nuclear substance? We know the chemical elements of which they are composed, but we do not know as living substances the physical and chemical laws which they follow. The mainsprings of life, however, rest there. Broadly speaking, our increased powers of observation with the microscope have brought us so far, and it is reserved for chemical and physical observation to lead us, if possible, still deeper into the secrets of the cell. Meanwhile, what I would desire to emphasise is the noteworthy achievement of a cellular morphology and physiology. The identity in substance, the identity in structure, and the identity in essential functions all rest on a common basis. We must, however, not be led to regard protoplasm as a definite chemical conception, or as being in any way a definite chemical body; its complex and varied activities put this out of the question. As to these

inner secrets of its structure and behaviour we know next to nothing. We have noted that cell function expresses itself essentially in the phenomena connected with growth, nutrition, reproduction, sensation, and motion, and these were discussed broadly in the previous series of lectures on the Cell as the Unit of Life. It is now my intention to take up various phases of these activities in connection with the cell, and to discuss the more important of them in greater detail. The cell consists essentially of organic, of proteid, matter, and its vital activities centre on metabolism in the weaving and unweaving of the molecules constituting its organic substratum. The cell is a laboratory which is fully equipped with all the agents necessary for the work it has to do. The subject of this course has been stated as 'Cellular Physiology, with special reference to the Enzymes and Ferments,' and will, therefore, exclusively deal with the functions of the cell in relation to the production of enzymes and with the cell as the originator of fermentation processes. We must first endeavour to understand what is usually meant by the term 'fermentation'—in other words, the nature and the cause of the processes to which this term has been applied. The living cell is in responsive touch with its environment; external physical factors, such as light, heat, and moisture, act as stimuli to its vital activity. To these the cell responds, and if there is a normal balance between these external factors and the cell itself as regards action and reaction, we have the cell unfolding all its normal attributes. Whatever functions of a general or special character it may possess, the cell requires food wherewith to build up its framework or structure and to gain the

necessary amount of energy. The matter and energy are acquired from the outside world. The matter and energy so acquired are not destroyed, although transformed into other kinds of matter and energy; there is a conservation of both, and they are ultimately restored in some form or other to the environment of the cell. The cell produces chemical changes in its material environment, and manipulates the material of the inorganic and organic world for its own special purposes. The presence of suitable food acts as a stimulus on the cell, and one of the most interesting phases in its life history is that which is connected with its processes of nutrition. We will see this more clearly in our study of fermentation. What is fermentation? And what are the reasons that have led to our regarding such a process as a form of cellular activity, thus bringing the process within the sphere of the cellular doctrine just stated? There is no line of investigation which has proved of such wide-reaching influence as the research which has been made upon the nature of fermentation. It was the researches of Pasteur and many distinguished chemists with regard to fermentation processes which led to the foundation of a biological doctrine concerning them, and to the establishment of the germ theory of disease as well as to the great practical achievements of Lister and many other eminent men.

A popular acquaintance with fermentation processes is of very ancient date, going back as it does to the early acquaintance of mankind with the intoxicating properties of the juice of the grape. Indeed, for a long time fermentation and alcohol production were practically synonymous terms. If the nature of the process was

unknown, the process itself was well known and appreciated. The name is probably due to the gas evolved in the production of the spirit, which causes the fermenting liquid to froth or foam and to be in a condition of agitation or unrest. This observation led in earlier times to some confusion through the application of the term *ferment* to processes of chemical action associated with the development of gas, such, for example, as occurs on the contact of chalk with an acid. The term was seized upon by the alchemists, and they sought to explain the sundry chemical processes observed by them by the action of a body termed *fermentum*. There was in all this a confusion of inorganic and organic chemical processes. As regards the alcoholic fermentation, various opinions were put forward from time to time. Thus about the fifteenth century the alcohol was considered to exist pre-formed, and the fermentation to consist in a purification of this pre-existing alcohol from the various impurities that were attached to it. In the sixteenth century distinctions came to be drawn between processes of digestion and processes of fermentation, and the suggestion was made that putrefaction was closely related to fermentation. The difficulty, however, remained of drawing a distinction between genuine fermentation processes and those which only resembled them in one external feature—viz. the development and the escape of gas.

In the seventeenth century the distinction was first noted and drawn as regards fermentation between the *gas* that was produced and escaped from the fermenting liquid and the permanent product, the *alcohol* that remained behind. The identification of this 'gas vinorum' with

carbonic acid was likewise made. A further advance was made when the distinction was drawn between the *gas* produced in the course of a fermentation and that produced in the decomposition of carbonates by acids, the former being stated to be a process of decomposition and the latter a process of combination. Still further advance was made in the observation that only *sweet* or *saccharine* fluids underwent this peculiar fermentation, and that the alcohol is a *newly* formed body, a *product* of the fermentative process. Becker came to the important conclusion that combustion and fermentation are analogous processes; he concluded that for both processes air is necessary. Further, a distinction was drawn between the genuine fermentation process in the narrow sense of the word, a process which resulted in the production of alcohol, and the acid fermentation in which vinegar was produced. The external and the main results of the process came thus to be fairly recognised. Towards the end of the seventeenth century Stahl devoted a deeper study to such processes, and formulated a startling hypothesis considering the elementary knowledge common to his time. 'A body which is undergoing decomposition, say putrefaction, produces very easily the same kind of change in another body. A body undergoing such movements may very easily produce similar movements in one which is in a state of quiescence or rest.' Here we have an indication of the infective action of fermenting liquids. Boerhaave concluded that only vegetable matter underwent fermentation, whilst animal matter in the course of its break up underwent a process of putrefaction. We have therefore reached a stage where there was a tolerable

clearness of view and a recognition of certain great classes of fermentation, such as the alcoholic, the acid, and the putrefactive fermentations.

We now come to the age of Lavoisier and the discovery of the significance of oxygen not only in all chemical, but likewise in all vital processes. The exact study of chemistry commenced, as well as a development of quantitative chemical methods, with the aid of the balance. Such observations applied to the fermentation of saccharine fluids showed that as a result of the process sugar disappeared, whilst alcohol and carbonic acid appeared as newly formed bodies—*i.e.* the sugar had undergone a chemical decomposition into alcohol and carbonic acid. The fluid was likewise found to contain a small quantity of acetic acid. The sugar was therefore split up into two parts: the one becoming oxidised to form carbonic acid, the other being converted into alcohol which likewise contained oxygen. Lavoisier's attention was entirely occupied with the chemistry of the process—he was not concerned in his investigation with the cause—and the same applies to the further inquiries of Gay Lussac. Various attempts were made by others to explain the process as being due to a catalytic or contact action, as well as by chemical and electrical theories. We have, at any rate, at this stage reached a knowledge of the chemical process in the alcoholic fermentation, and that it consists of a decomposition of saccharine matter into alcohol and carbonic acid. In addition there are various by-products, such as glycerin and acetic acid. Can we explain the process as being a purely chemical one? The alcoholic fermentation is accompanied by the development in the fermenting liquid

of a *distinct* deposit either as a sediment or as a scum. Leeuwenhoek in the seventeenth century examined this deposit with the aid of his magnifying glasses and found it to consist of small bodies of a spherical or globular shape, but their nature he was unable to determine. In the eighteenth century they were supposed by some to be of an animal and by others of a vegetable nature. One fact which was long recognised was that decomposable organic substances, such as foodstuffs, if boiled and kept from contact with the air, remained sweet and unchanged. The explanation of this fact was not, however, given till a much later date—viz. that it is due to the destructive action of heat upon the living germs of putrefaction. The period immediately following Lavoisier has been termed the age of oxygen, and the explanation of the keeping properties of food and other decomposable matter after boiling was at that time thought to be due to the driving out of the oxygen which would otherwise have started a fermentation or decomposition process. It was believed that the presence of oxygen was the necessary condition for the occurrence of a fermentation. A purely chemical conception therefore prevailed with respect to fermentations at the beginning of the nineteenth century. The clue was, however, eventually found in the old observation of Leeuwenhoek with reference to the spherical bodies he had detected in fermenting liquids. Erxleben (1818) suggested that these globules—*i.e.* the yeast cells—were vegetable organisms, and that they produced the fermentation. Cagniard Latour (1835), as a result of his studies, concluded that the yeast cell was a vegetable organism, and that to it the alcoholic fermentation is due. And

somewhat later Schwann made the same discovery. Schwann controverted the theory that the air as such or the oxygen it contained was responsible for the fermentation process, for he found that air which has been heated but otherwise chemically unaltered did *not* induce a fermentation if certain conditions were observed. Schwann in his experiments observed that fermentable fluids, after being boiled, fermented, and organisms developed in them when *ordinary* air was introduced, but that *no* fermentation occurred if the air admitted had previously been subjected to a strong heat. There was therefore a *something* present in ordinary air which led to the decomposition of the fluid by organisms. It was consequently a justifiable conclusion that the something present in ordinary air and which disappeared in heated air consisted of the germs of organisms. Further, it might be that specific organisms were closely related to specific decomposition processes, and might be the cause of the same—at any rate, the mere presence of oxygen was *not* the deciding factor in such processes. We have, therefore, the possibility emphasised of the intimate relation between the development of certain organisms or vegetable cells and given decompositions or fermentations. Something, at any rate, had been destroyed by the heat in Schwann's experiments.

Many arguments were brought forward against this view, and the older theory practically prevailed until the publication of Pasteur's classical researches. The chemists strongly opposed the vital theory, and the distinguished chemist Liebig published an ingenious theory with reference to fermentation. This theory was based on molecular mechanics (*i.e.* upon the vibrations common to molecules)

and asserted that all fermentations were the result of the transmission of molecular movements which were produced by a chemical decomposition of the substance causing the fermentation. Thus the alcoholic fermentation was carried on by the decomposition of the yeast, and the fermentation of proteids by the decomposition of pepsin, and so on.

In this way Liebig regarded the decomposition of the ferment as the cause of the decomposition of the given substratum; according to him there is a breaking up of ferment and fermentable substance. Thus sugar is a body of some stability, but held together by very slight chemical forces, yeast is an unstable and easily decomposed organic body, and this instability it transmits to the sugar. Fermentation is a special form of putrefaction in which the fermenting substance is non-nitrogenous. Liebig's theory was, therefore, purely chemical and physical, and disregarded the vital activity of the yeast cell. We have mentioned Schwann's experiments, which showed that heat destroyed something essential to the fermentation process. Schulze likewise found that if the *air* was first passed through strong sulphuric acid, which has a destructive action on organised matter, *no* fermentation occurred. It was further found that a simple mechanical filtration of the air through cotton wool was sufficient to rob it of all fermentative power. Heated air and filtered air *cannot* produce any fermentation. This all pointed to a vital interpretation of the process, and the conclusive proof was furnished by Pasteur. Instead of wool Pasteur used a soluble filter such as gun-cotton, through which the air was filtered. The filter was then dissolved in ether, and the undissolved remainder, representing the detained solid

matter which had been suspended in the air, was collected and examined. In this deposit various forms of organisms were detected. Further, series of flasks containing boiled fermentable fluids were each connected with a tube which had been strongly heated. The result was that only heated air passed into the flasks, which consequently remained undecomposed. These are his experiments in their simplest form, and we need not refer to the more complicated devices which Pasteur employed.

Wool plugs containing the filtrate from the air gave rise to vegetative growths, and likewise started fermentative processes. The infecting principle in the air consisted of solid particles which could be filtered off and which were readily destroyed by heat. Under the microscope they appeared to be organised bodies, and by sowing them in suitable fluids the same changes were produced which occurred on the introduction of ordinary air. In the words of Pasteur, 'There are always present in the air organised bodies, which cannot be distinguished from the germs of the infusion organisms. If one sows the organisms in previously boiled fluids, the same organisms appear in the fluids as develop when these are exposed to free access of air. Their distribution varied; thus flasks opened on the summit of a mountain remained sweet, whilst flasks opened in a dwelling became putrid.'

There is always to be found in a fluid undergoing an alcoholic fermentation a corresponding growth of the characteristic yeast cell. The yeast cells are not generated spontaneously, they are derived from previously existing yeast cells. Despite many criticisms, Pasteur's researches, and the vital theory based on them, were accepted,

corresponding as they did to all the observed facts. The upholders of the vital theory made the following postulates : 'That in all cases where vegetating yeast organisms are to be observed and sugar is present, the sugar splits up according to the formula of the alcoholic fermentation. That in all cases where such a fermentation occurs, living yeast cells are likewise to be found.' Thus both phenomena are most intimately connected. In other words, the alcoholic fermentation is a physiological act of the organisms in question. The alcoholic fermentation consists in a decomposition of sugar into alcohol and carbonic acid and some other bodies, and this is paralleled by a corresponding growth of yeast cells in the mixture. The seeding of a very small quantity of these cells in a suitable fluid is sufficient to start the alcoholic fermentation, whilst the process cannot be started by the addition of any merely chemical body. All factors which prevent the development of yeast, which kill it or which exclude it from the sugary fluid, stop or prevent the fermentation. The vital theory applies not only to the alcoholic, but to other kinds of fermentation, such as putrefaction. And no one has been able to demonstrate a spontaneous generation of the living agents in this or in any other fermentation.

I have dwelt on the alcoholic fermentation, because none has been so carefully investigated and because the results obtained by its study have been of the widest significance not only in the chemical, but most notably of all in the biological field. This fermentation, as I have said, has been demonstrated to be a physiological act on the part of a self-multiplying vegetable organism—the

yeast—a minute quantity of which is capable of starting the process on account of its extraordinary power of self-multiplication. This may to us seem an obvious statement; but to appreciate its full significance we must transport ourselves to the time of Pasteur's earlier work and consider the ideas that then prevailed in order to appreciate fully the significance of this discovery, which ushered in a new and fruitful era of research, culminating in our own times in the germ theory of disease.

Not only has the biological nature of the alcoholic fermentation been established, the same fact has been proved with regard to a large number of other fermentative processes; they are likewise biological acts on the part of living infective agents. We may quote as examples the production of vinegar from alcohol, the leavening of bread, the curdling and the souring of milk, the production of wines, the fermentations occurring in tanning processes and in the preparation of tobacco, the fermentation of cellulose, and so on. In all these living vegetable cells are at work, whether of the nature of yeasts or of bacteria. A large amount of research is at present engaged with the study and isolation of the various agents at work in these various fermentations. The results are of great technical importance, as the possibility is now opened out of utilising pure cultures of these various organisms in various industries in which micro-organisms play an important *rôle*. The greatest achievement in this respect has been in the isolation and utilisation of pure cultures of the essential yeasts in the brewing industry. You will, therefore, see that the study of the physiology of the unicellular organisms is of great practical

importance, and that the investigation of this promising field is now proceeding on scientific lines. Let me give an instance of the old rule-of-thumb and the new methods. In China and Tonkin a spirit is prepared by means of so-called 'rice balls,' which are bought by the natives for the purpose. There is a Chinese receipt for the preparation of these rice balls, which mention, I think, about twenty different substances to be used for their preparation, such as sundry essences, &c. ; but of a living agent no mention is made, the virtue resting, as the Chinese believed, in the cunning compounding of various substances which, when added to rice, produce an alcoholic liquor. It was reserved for a French investigator, M. Calmette, to examine the process, following Pasteur's methods. M. Calmette found that this *mixtum compositum* was of absolutely no value, but that the active agent was an organism attached to the husks of the rice grains scattered through the mixture, and which was therefore unwittingly introduced into the ferment ball in the course of its preparation. This organism was a species of mould which converted the rice starch into sugar, which was then in its turn converted into alcohol by various species of wild yeasts. M. Calmette obtained pure cultures of this mould, which, when associated with a pure culture of a yeast, produced, though in an improved and purer form, the same alcoholic fermentation. It is interesting to note that this organism, obtained in Tonkin, is now busily engaged in manufacturing alcohol in French distilleries. This is as striking an instance as any of the aid that may be furnished by exact research to industrial processes. I am afraid that in this country the appreciation

of the practical bearings of scientific research is not so keen as it is abroad, where every help and encouragement are given to the scientific worker. The result is that it is from the Continent we are learning the principles of scientific brewing; and Germany has recently been showing us how to make indigo.

We have proceeded so far in our inquiry with regard to fermentation processes as to be able to say that they mainly consist in a decomposition of organic substances through the action of cellular organisms which may be of the nature of yeasts, of fungi, or of bacteria. The ferment agent in the course of a fermentation is an organised living cell. This will explain what is meant by the term *organised ferments*, which is commonly applied to these living agents. There are, however, certain ferment activities which appear to be independent of the living cell and which are capable of running their course without the direct intervention of the cell itself. The cell, that is to say, does *not* produce the chemical changes by its *direct* action, but indirectly by means of a substance which it forms and which it secretes. Such a substance can act quite independently of the living cell which produced it. This form of chemical action is known as enzyme action, and the active substance is known as an enzyme. These enzymes are widely produced by the cells of plants and animals, and they have been termed *unorganised ferments*. Hence the distinction made of the ferments into two groups—the organised and the unorganised ferments. The plant cell furnishes one of the most typical instances of the production of an unorganised ferment or enzyme in the substance known as *diastase*.

It was found that germinating barley when added to starch paste liquefied it, and that as a result sugary matter was formed. If a watery extract was made of the barley grain, and alcohol was then added to the watery extract, a precipitate occurred. This precipitate, when redissolved in water, converted starch paste into sugar. The process was regarded as a fermentation, and to the ferment the name *diastase* was applied. It differed from an organised ferment such as the yeast cell, inasmuch as it was of an amorphous character and could be precipitated without losing its active properties, which are exercised without the intervention of the living cell which had produced it. On account of these properties it was termed an unorganised ferment. It was observed that the liver possesses properties similar to those of germinating barley, and that a diastase can be prepared from it; whilst the gastric juice was found to contain a substance which dissolved the nitrogenous constituents of the food and thus rendered them diffusible. To this substance the name 'pepsin' was given. The pepsin is a secretory product of the cells of the mucous membrane of the stomach. It can be precipitated from its solutions and can be directly extracted from the gastric cells by means of a solvent such as glycerin; it belongs therefore, like diastase, to the group of the unorganised ferments. These instances of unorganised ferments or enzymes will be sufficient for our present purpose. A large number of such enzymes have been isolated from animal and plant tissues and will be referred to in subsequent lectures. They play a most important part in the life of every cell.

The salient points in our general survey so far have been in the first instance the recognition of the biological nature of fermentation processes, and, in the second instance, the classification of the ferments into two great groups—the organised and the unorganised or soluble ferments. The organised ferments are such in which the direct agent is a living organism, and the process occurs as a result of its growth and multiplication. The unorganised ferments are not living cells, but products of the same, and are capable of producing a ferment action quite independently of the cells from which they originated. The general term applied to these unorganised ferments is that of *enzymes*.

This is the classification of the ferments that has been most generally adopted, but it is not a convincing or a final one, as will be seen in the course of our subsequent considerations.

The study of fermentation processes has brought us once more back to the intimate study of the cell and its activities, and the study of the enzymes or ferments is one of the most important branches of Cellular Physiology. If we consider the great processes that are going on around us in Nature and involving the redistribution of the various forms of dead organic matter, we find that they are biological acts due to the activity of living agents. Dead plant tissues are mainly attacked and decomposed by unicellular organisms. Substances such as cellulose, which under ordinary circumstances are most resistant to the action of purely chemical agencies, are successfully attacked and disintegrated by bacterial cells. Further, dead animal matter undergoes in the process of putrefaction a resolution

into simpler chemical compounds, which can once more be taken up and utilised in the cycle of life. In these processes the main agents are bacteria; to use the old expression, living ferments are at work. We have seen that such living ferments produce the fermentation of saccharine fluids, for example, the yeast cells, whether the result be beer, wine, or spirit. The agents in all these cases are free-living, independent unicellular organisms. And in the *fixed* cells which constitute the tissues of plants and animals similar processes occur. In the germinating barley diastase saccharifies starchy matter, and in the saliva of animals a like process occurs. Amongst the insectivorous plants albuminous matter is digested, whilst the gastric juice of animals performs a similar function. All these processes are physiological acts on the part of living cells. This cardinal fact having been established, the *mode of action* of the cell must be more closely studied. It has long been held that in the alcoholic fermentation, for example, the process is due to, and cannot proceed without, the direct intervention of the living yeast cell. Hence the distinction drawn of *organised* ferments—the organised ferment in this case being the yeast cell. In the case of other fermentations, such as are due, *e.g.*, to diastase in the plant and to pepsin in the animal, the ferment action is the result of a cell secretion, of the nature of ‘an *unorganised*’ ferment. This distinction, however, is not one that is of a satisfactory character, or that is likely to be of a permanent nature. It is a more or less arbitrary distinction drawn between processes according as they happen to occur *inside* or *outside* the cell—a distinction between processes of an intra- or an

extra-cellular nature. It is difficult for us to conceive that bodies of the nature of enzymes are not produced which act *within* the cell itself and not simply as secretion products. And as a matter of fact it is not easy to draw a hard-and-fast line between the so-called organised and unorganised ferments—they grade into each other. The ferment action in every case appears to depend on a something which is a derivative or product of the cell protoplasm, but the relation of which with and intimacy to the cell protoplasm varies. We may have the ferment passing out of the living cell in the form of a secretion and acting independently of the cell protoplasm, as in the case of the large group of the soluble ferments or enzymes. And such ferments or enzymes may equally readily be extracted from the cell. We may, on the other hand, have the ferment body *anchored* to the cell protoplasm, so that it has every appearance of coming under the rubric of the insoluble or organised ferments. An example of this is *invertase* as it occurs in the yeast cell; it is not a secretory product of the yeast cell. But if the vitality of the yeast cell be impaired, if the cell be triturated or killed, the ferment passes out of the cell into the surrounding medium and evinces its characteristic action—in short, it behaves exactly like a soluble ferment or enzyme. And, further, the great typical example of an organised ferment—viz. the alcoholic yeast ferment—has always been regarded as indissolubly bound to the living protoplasm. Yet by employing drastic methods of cell disintegration Buchner claims to have succeeded in isolating the alcoholic ferment and to have demonstrated its action apart from the living

cell. To this ferment or enzyme Buchner has given the name *zymase*. There are other ferments which, if they exist in the living cell, have not yet been isolated, as, *e.g.*, the lactic-acid and the acetic-acid ferments, but as the result of Buchner's work one may regard this as a possible achievement. The view is gaining ground that fermentation is not directly bound up with, or to be regarded as identical with, vital action, but that it is an effect of subsidiary agents of the protoplasm, which may be set free from or may remain anchored to the cell and its protoplasm. These, however, still remain theoretical considerations, to which we will return in a subsequent lecture. We have, nevertheless, said enough to show the difficulty that exists in sharply dividing ferments into the organised and the unorganised.

The ferments which will mainly occupy our attention in the present course of lectures are those known as the *Soluble Ferments* or *Enzymes*. The enzymes, as we have already indicated, are widely distributed, and one may say ubiquitous in plant and animal life. In the case of the animal they are mostly formed in the cells of special organs or glands, and are secreted. Thus the cells of the salivary gland secrete a saccharifying ferment and those of the stomach a proteolytic ferment, whilst the intestinal glands secrete ferments of varied action. Their secretion normally occurs under the effect of the stimulus given by the presence of food. Amongst plants we note everywhere the presence of soluble ferments or enzymes. In the complex plant there appear to be special cells or groups of secretory cells which are concerned with their formation. The ferment appears to exist in the cells in

an inactive form, or *zymogen*, as it has been termed, which first becomes the active enzyme on passing out of the cell. The universal distribution of these enzymes is sufficient to indicate their importance in Cellular Physiology. The plant and animal do not obtain their essential food in a directly assimilable form. The food must first be converted into diffusible bodies before it can be taken up by the cell protoplasm, and this is one of the peculiar, if not the main, functions of the soluble ferments or enzymes. In the plant the reserve substances which it stores are rendered available when required by the action of various enzymes; whilst as regards the embryo plant it comes into the world with a store of reserve material upon which it lives until it is sufficiently developed to lead an independent existence and to absorb nutriment by the aid of its roots and leaves. In this instance likewise it is through the action of enzymes that the reserve substances are rendered available for the needs of the infant plant. Indeed, the enzymes appear to be essential factors in the life processes of any organism, whether it be plant or animal. As to the exact nature of the enzymes there is little to be said, as they have not been isolated in a pure form; it is by the specific effects they produce that we are enabled to differentiate them. Their classification, therefore, is based not upon their nature, but upon their mode of action, or, rather, upon the nature of the matter upon which their specific action is exerted.

LECTURE II.

Classification of Enzymes—Conditions Modifying the Activity of Enzymes—Nutritive Requirements of Living Organisms—Examples of Various Classes of Enzymes and their Actions—Bacterial Toxins—Diphtheria—Tetanus—Antitoxins—Immunity.

Having now surveyed some of the phenomena connected with the vital activities of protoplasm and of cells, the processes of fermentation as exemplified in the production of alcohol from saccharine matter by yeast, and the occurrence of ferments, organised and unorganised, we may now pass on to consider more in detail the unorganised ferments or enzymes.

As to the exact nature of these enzymes little can be said, as they have not yet been isolated in a pure form. It is by the specific effects they produce that we are mainly able to differentiate them. Their classification, therefore, is based *not* upon their exact *nature*, but upon their *mode* of action on different groups of substances. Looked at from this point of view the enzymes or soluble ferments may be broadly differentiated into six great groups. We have—1. The *proteolytic ferments*, which convert insoluble organic substances of the nature of proteids into soluble modifications, a typical example being the pepsin as found in the gastric juice. 2. The

clotting enzymes, such, for example, as produce a curdling of milk, a typical example being rennet, which converts the milk into a clot and a watery fluid, *i.e.* into a solid curd and a fluid whey. This ferment is employed in the manufacture of cheese, the clot or curd consisting of the casein of the milk, and representing the crude cheese. 3. The *saccharifying enzymes*, which transform insoluble bodies of the nature of starches into soluble sugars; a typical example is diastase as found in the plant. 4. The *glucoside ferments*, which decompose complex substances existing in plants into sugar and other bodies, a typical example of which is emulsin, as it exists in the kernel of the almond. 5. The *oil and fat ferments*, whereby fatty matter undergoes decomposition with the liberation of free fatty acids, *e.g.* the lipase, as found in the digestive tract of animals. 6. The *oxidases*, or oxidising enzymes, which assist in the oxidation of various substances, as, *e.g.*, laccase, which appears to be concerned in the production of lacquer varnish from the sap of the lac tree in Asia. It will be seen from this classification that the action of the soluble ferments or enzymes is of a varied character, and that the action of these enzymes is in so far of a specific character, as in each case it is confined to a decomposition of a certain class of chemical compounds. The proteolytic enzyme, *e.g.* the pepsin, whilst acting on proteid does not act on starchy matter, and the saccharifying enzyme, *e.g.* the diastase, whilst acting on starchy, is inert as regards proteid, matter.

These enzymes are in all cases prepared by the protoplasm of the cells and secreted for special purposes, of which one of the main objects is to carry out the work of

digestion. Whilst a minute quantity of such an enzyme is capable of setting up extensive change in a large amount of a suitable substance, the enzyme itself does not appear to undergo any chemical change or to be used up in the process.

The enzymes, in addition to the presence of suitable material on which to exert their action, require a certain stimulus in the form of *heat*, and for each enzyme there is a certain temperature—an optimum temperature—at which it develops its maximum activity. This temperature, in the case of the animal enzymes, is about that of the body temperature. If the temperature is raised above the optimum amount, the action of the enzyme is weakened; and if the temperature is raised to a degree approaching the boiling point, the enzyme is destroyed and its action entirely ceases.

Light likewise has an injurious effect upon the enzymes, and produces their gradual decomposition.

As I have said, the enzymes are the main agents in digestive processes on the part of the cell. This digestion may either be effected on reserve food material stored within the cell itself, as in the plant, or it may take place in connection with the absorption of food from without.

What is the nature of the special foods which an organism requires for the maintenance of its life processes? The most important of such foods are the proteids, inasmuch as the cell *cannot* exist without them, nor can they be replaced by any other form of nutritive material. The proteids are essential constituents of the protoplasm of every cell, whether it be animal or vegetable.

All proteid bodies have this in common, that five chemical elements enter into their constitution—viz. carbon, hydrogen, oxygen, nitrogen (hence the name nitrogenous bodies), and sulphur. The proteids are further distinguished by the fact that they never occur in a form of complete solution, and consequently they are not diffusible—*i.e.* they will *not* pass through a membrane whether it be a parchment one or the membrane surrounding a living cell. The proteids further, on boiling, are usually converted from a liquid or soluble form into a solid, insoluble, or coagulated modification: they are coagulable bodies.

Whilst the plant cell is able to produce a synthesis of its proteid substance from inorganic material, such as salts of ammonia, the nitrates, sulphates, etc., the animal cell is unable to do this. In the case of herbivorous animals the necessary proteids for their vital processes are derived from vegetable food; whilst in the case of carnivorous animals they are derived from the meat or other constituents of a mixed diet. As regards the animal, the proteids which it requires are those which are able to yield soluble or diffusible bodies called peptones. There are two other important groups of organic foodstuffs, viz. the fats and the carbohydrates, which, in contradistinction to the proteids, contain neither nitrogen nor sulphur; they are simply composed of the three elements, carbon, hydrogen, and oxygen. The *fats* are widely distributed in the animal and vegetable kingdom, and are to be met with in the seed, fruit, and root of the plant, and in animals in the subcutaneous tissues; whilst unicellular organisms, such as the tubercle bacillus, may contain as much as

40 per cent. of fatty matter. They constitute one of the main sources of heat in the animal body, are decomposable and are soluble in ether. The *carbohydrates* form the greater part of the solids in plants, just as the proteids form the greater part of the solids in animals, and are mainly stored up as starch in the plant and as glycogen in the animal. They are readily converted from this insoluble condition into soluble bodies or sugars.

These are the main types of organic substances which the cell requires for the purpose of its nutrition.

We are not, of course, here concerned with the salts or inorganic constituents of the food.

Digestion of food is a chemical change which is produced in the food, and this change is brought about by various digestive juices produced by the cells of the living organism. These juices contain a certain body, of the nature of a soluble ferment or enzyme, which is the active agent in bringing about the change in the food. How completely equipped the living organism is for carrying on these processes we can best illustrate by the process of digestion of a mixed diet as occurs in man. The digestive processes in man are carried on in the mouth, the stomach, and the intestine. This digestion has, as its object, the separation of the useful from the useless constituents of the food, and the conversion of the former into a condition which renders their absorption into the living organism possible, as well as their consequent utilisation for the various purposes of the organs and cells.

The digestive changes begin in the mouth; the first fluid with which the food comes in contact is the *saliva*, which is a colourless fluid and has an alkaline

reaction. It acts on the starchy constituents of the food, converting them into soluble, sugary substances. This it does in virtue of an enzyme secreted by the salivary glands named ptyalin, or, as one might term it, *salivary diastase*. In the stomach the food comes in contact with a second digestive fluid, which is distinguished by its acid reaction, due to the presence of free hydrochloric acid. As a result, the nitrogenous or proteid food constituents are converted into peptones, in virtue of the action of a soluble enzyme, called pepsin, a proteolytic ferment. There is a second ferment in the gastric juice, the chymosin, or rennet-like ferment, which produces a curdling of the milk or of the casein which it contains.

Passing from the stomach to the intestine the food comes into contact with other digestive juices, which have an *alkaline* reaction, and in the secretion of the pancreas we have a juice which acts on all the different forms of foodstuffs and renders them absorbable, the proteids are peptonised, the starchy matters are converted into soluble carbohydrates, and the fats are split into glycerin and fatty acids. The pancreas therefore secretes, to use the exact terminology, a proteolytic ferment—*trypsin*, as it has been called—a saccharifying ferment, the pancreatic diastase, and a fat-splitting ferment, *i.e.* a *lipase*.

Of the six groups of ferments that we have defined, we find therefore representatives of four occurring in the human digestive tract.

We will now turn to an individual consideration of these various ferments, as regards their origin and mode of action, and in the first instance consider the *proteolytic enzymes*.

The proteolytic enzymes occur both in the animal and vegetable organism, and play an important part in the life processes of the cell. The proteolytic ferments are secretory enzymes which, without the direct intervention of the cell, convert proteid substances into simpler bodies.

The difficulties are great that lie in the way of an accurate study of the nature of these processes. Whilst the chemistry of the simpler carbohydrates and of their decomposition products is approximately understood and can be studied, we know little or nothing of the chemical structure of the proteid bodies, so that it is impossible to follow the changes they undergo in an exact chemical fashion, or to represent these changes by a chemical formula. It is, therefore, the end products of the fermentative process which one has mainly been able to isolate and about which we know most.¹

The proteolytic enzymes may be divided into *three* groups. Of the *first* group the most typical representative is the pepsin of the stomach, as well as other analogous ferments. They convert proteids into albumoses and peptones, and are generally only active in faintly acid solutions.

Of the *second* group we have a typical example in trypsin, and its analogues, which act in neutral or faintly *alkaline* solutions. They produce a more energetic

¹ Since this was written the researches of Professor Emil Fischer have shed considerable light on the constitution of the proteid molecule. Whether the cleavage is affected by means of digestive ferments or by chemical reagents, the general order of the cleavage is the same, resulting in the production first of proteids of smaller molecules than the original native albumin, the proteoses or albumoses, then of still smaller molecules, the peptones; later the peptone breaks up into simpler bodies, the polypeptides (many of which have been synthesised or built up chemically by Fischer), and finally into amino-acids.—ED.

decomposition of the proteid substances, and as a result simpler derivatives of the same, such as the amido-acids and various crystallisable bodies.

The *third* group, acting on proteid bodies, are the *clotting ferments* to which we have already referred, in the typical example of the rennet ferment.

In animals these ferments are secretions of the glands of the digestive tract and are to be found in its juices, and their action is usually confined to certain portions of the digestive tract. The existence of a peptic ferment in the gastric juice was long suspected. Schwann, as the result of his researches, came to the conclusion that a ferment was the cause of gastric digestion, and to this ferment he gave the name *pepsin*. He showed that it was a special, a specific, product of the mucous membrane lining the wall of the stomach. Whilst the existence of this ferment has been demonstrated it has not been possible to isolate it in a chemically pure condition. By the use of precipitating agents, such as alcohol, a precipitate can be obtained which contains the active ferment, and, further, the enzyme is soluble in glycerin and can be extracted from the gastric mucous membrane by means of this agent.

The pepsin does not appear to exist in the cell as the active ferment, but in the form of a pro-ferment or zymogen stage, and in this instance the zymogen has been termed *pepsinogen*. Dilute acids quickly convert this pepsinogen of the cell into the pepsin enzyme.

Pepsin is to be found in the stomach of almost all vertebrate animals, and it is probable that the digestive enzyme of some insectivorous plants is a pepsin. The

most active pepsin is that to be found in the gastric juice of the dog. In warm-blooded animals it does not exert its action at temperatures below 10° C., but it has been stated that the pepsin of the frog, for example, remains active even at freezing point. The pepsin appears as a white or yellowish amorphous substance, and is soluble in glycerin, in water, and in dilute acids, and can be precipitated from its solutions by an excess of alcohol. Its action is destroyed when its solutions are heated to 55° to 70° C. It appears to form a loose combination with hydrochloric acid.

Let us consider its action as seen, *e.g.*, in the gastric juice of man. The first thing that strikes us is that the digestive action on proteids is carried out in an *acid* medium. This acid reaction is due to the presence in the gastric juice of free hydrochloric acid. The general view has been that it is the presence of this hydrochloric acid which renders the action of the pepsin on the proteids possible and their consequent conversion into soluble and non-coagulable bodies, the peptones. This has not, however, been considered by many observers a satisfactory explanation. They would consider that the main function of the free hydrochloric acid in the stomach is to act as an antiseptic—*i.e.* to kill the micro-organisms which pass into the stomach along with the food, and which in the course of their development in the digestive tract would tend to produce decomposition of the food previous to its absorption into the general system. Bunge states that as a matter of fact the amount of hydrochloric acid present in the gastric juice (0.3 per cent.) corresponds exactly to the amount which is necessary to prevent the

development of fermentative organisms. And, as a matter of fact, it is true that under normal conditions no marked fermentative changes occur in the stomach; whilst in conditions of disease, accompanied by lessened secretion of the gastric juice, there may be a marked occurrence of fermentation and putrefaction along with the development of gas, as a result of the action of micro-organisms. This antiseptic action of the gastric juice was noted one hundred years ago by Spallanzani, who found that pieces of meat when covered with gastric juice did not undergo putrefaction. It is likewise a remarkable fact that a number of the lower animals, such as certain snails or slugs occurring in the sea (*Dolium galea*), possess at the upper part of their digestive tract glands which discharge a secretion rich in free mineral acids, but which secretion contains no pepsin or digestive ferment, and in this respect is absolutely inactive or without digestive power.

These secretions contain over 2 per cent. of sulphuric acid and as much as 0.4 per cent. of hydrochloric acid. This is a remarkable fact, the secretion of strong free mineral acids by these animals as well as in the course of gastric digestion—*i.e.* a separation of acids from alkaline tissues. The following theory of the origin of hydrochloric acid has been given. The free carbonic acid which is present in the blood sets free a small quantity of hydrochloric acid from the sodic chloride of the blood, and this is taken up and discharged by the epithelial cells.

That the hydrochloric acid is secreted to aid the pepsin in its digestive action has not been proved with sufficient accuracy, though there can be little doubt that its antiseptic properties as regards the food during its

sojourn in the stomach are of great importance and value.¹

We have no chemical test for the presence of pepsin in a digestive fluid beyond the testing of the action of the juice on proteid substances, such as fibrin or egg albumin. The pepsin itself is not consumed in the course of peptic digestion, and its action takes place best at about the temperature of the body. All genuine proteid bodies are attacked by the pepsin, and the result is a decomposition into bodies of simpler molecular constitution. The cardinal feature is that the non-diffusible proteids are converted into diffusible bodies—the peptones. The process is essentially a hydrolytic one, a process of hydration, and is analogous to the hydration of proteids when they are submitted to the action of moist heat above the boiling point of water or to the action of purely chemical agents, such as dilute acids. It is impossible as yet to follow chemically the changes that occur in the transformation of the proteid molecule into the end product—the diffusible peptone. It has, however, been demonstrated that there are intermediate stages and transition bodies formed. It would carry us too far to consider the results obtained by research in this direction in any detail. To these intermediate substances which occur as transition bodies between genuine proteids and the genuine peptones the term *albumose* or *proteose* has been applied.

The end products, the peptones, are not coagulated when their solutions are heated. The digestion of proteids in the stomach is a chemical process proceeding quite

¹ A. Macfadyen, *Journ. Anat. and Physiol.*, xxv., p. 419.

independently of the living cells through the action of the pepsin in the presence of hydrochloric acid. The fresh gastric juice under suitable conditions acts just as well outside as inside the body. If, therefore, we obtain the essential constituent, the pepsin, by precipitation or extraction from the gastric juice, its action can be studied in the test-tube, and in this way numerous important results have been obtained as to its mode of action, and likewise as to the relative digestibility of various articles of food. These experiments are readily carried out in the laboratory with an artificial gastric juice if certain conditions are observed. Favourite proteid substances for experimenting with are fibrin from the blood and the white of an egg. A solution is then made up of the pepsin, containing about, say, 0.5 per cent. of pepsin and about 2 to 3 per 1,000 of added hydrochloric acid. These are placed in a test-tube and the whole kept at a suitable temperature—*i.e.* about blood heat. This is ensured by placing the tubes in a beaker containing water, which is kept uniformly at the desired temperature by means of a gas regulator. Fibrin stained with carmine can also be used to render the effect more apparent. The action of pepsin can also be shown on gelatin, which it liquefies and changes into a gelatin peptone. The proteid matter when undergoing digestion swells, becomes transparent, and finally dissolves, forming the peptones, which are, as soluble bodies, capable of being absorbed by the cells of the digestive tract and being utilised after passing into the blood for the general nutrition of the organism.

We now pass to the consideration of the second type of proteolytic ferment or enzyme as found in

trypsin, which is secreted into the intestine by the pancreatic gland. It is, like pepsin, an unorganised ferment, and its action can therefore be studied independently of the living cells that produced it. Kühne showed that as a result of the action of the pancreatic juice a decomposition of proteids takes place which differs from that due to pepsin, inasmuch as other bodies are produced besides peptones—viz. leucin and tyrosin. The active ferment can likewise be precipitated from its solutions or directly extracted by solvents such as glycerin, but, like pepsin, it has not been obtained in a pure condition. Its action is most energetic in faintly alkaline solutions such as 1 per cent. soda. Trypsin-like ferments are very widely distributed amongst animals, and are amongst the most important enzymes for their life processes. The ferment, like pepsin, does not appear to exist in the cells as such, but in an initial or zymogen stage, which becomes the actual ferment on secretion.¹ Its characteristics are, as I have said, its action in an alkaline medium, a deeper decomposition of proteids than is the case with pepsin, and the appearance, in addition to soluble peptones, of crystallisable bodies, such as leucin and tyrosin, as well as of basic bodies. The pancreatic juice contains, besides *trypsin*, a diastatic and a fat-splitting ferment, but these need not detain us here. The trypsin produces a rapid liquefaction or peptonisation of gelatin.

Interesting observations have been made of apparently similar processes of digestion amongst unicellular organisms.

¹ This conversion of the pro-ferment trypsinogen into the active ferment trypsin appears to be brought about by the action of another ferment *enterokinase* contained in the *succus entericus* or secretion of the intestine.—ED.

Thus solid particles of food passing directly into the substance of the Amœba become surrounded by fluid so that they lie in a vacuole in the substance of the Amœba cell; in this cavity the food is digested and ultimately disappears. If the ingested matter be an alga cell, surrounded by a cellulose membrane, the proteid contents of the alga cell are digested, but not its cellulose membrane. This points to the action of some secretion on the part of the Amœba which can pass through the containing membrane and act on the proteid contents of the alga cell.

Artificial digestive experiments can likewise be carried out with artificial pancreatic juice, as in the case of the gastric juice. A solution of trypsin is prepared which has been rendered faintly alkaline, say, by the addition of soda. The best test objects for its action are either gelatin or fibrin, the mixture being kept at about blood heat. As the fluid is alkaline, bacteria readily develop in it and produce decompositions on their own account; the fluid therefore readily becomes putrid. For the proper study of an artificial trypsin digestion it is therefore necessary to prevent the development of bacteria, and this can readily be done by adding an antiseptic such as thymol or chloroform. This is always a necessary precaution in such experiments.

We have likewise active proteolytic ferments occurring in the vegetable kingdom. Thus the juice of the pineapple contains an active enzyme, to which the name of *bromelin* has been given, and which can be extracted from the pineapple juice. This proteolytic enzyme is associated in the pineapple juice with a milk-curdling ferment, just as is the case with pepsin in the gastric juice of

animals. The juice has a distinctly acid reaction. The active enzyme, the bromelin, if allowed to act on fibrin, produces leucin and tyrosin, as well as peptones, the ferment thus resembling in its action the pancreatic trypsin, and a similar action occurs on egg albumin.

The papaw tree's fruit has long been considered to have the property of rendering meat tender when cooked with it. As a matter of fact, the juice of the fruit has been found to contain an active proteolytic ferment. The active enzyme can likewise be extracted from the juice, and the name of *papain* has been given to it. In addition the juice, like that of the pineapple, contains a milk-curdling ferment. The papain acts best in a neutral or slightly alkaline solution. Its action—for example, on fibrin—is similar to that of trypsin, leucin and tyrosin being formed along with the soluble peptones.

In vegetable seeds which contain reserve stores of proteid material, proteolytic enzymes likewise occur. Thus Reynolds Green found that the seeds of a species of lupin contain an enzyme which is capable of digesting fibrin in a faintly acid solution, peptones resulting along with leucin and tyrosin, and a similar enzyme exists in the germinating seeds of the castor-oil plant; and other instances might be cited. Thus the juice of the common fig tree contains an active proteolytic enzyme.

In some instances a proteolytic enzyme is formed and secreted on the surfaces of plants, as in the case of the insectivorous plants which capture and digest insects. Thus the pitcher plants, *e.g.* the *Nepenthes*, attract insects which are drowned in the fluids they contain. The *Nepenthes* possess at the lower part of their pitchers glands

which secrete a proteolytic enzyme, and by its action the insects undergo a process of genuine digestion. It can likewise dissolve boiled white of egg, as well as fibrin. There are other plants which capture and digest small insects, such as the *Drosera*, which was investigated by Darwin in this connection. The leaves are provided with long stalked glands, which pour out a viscid secretion with an acid reaction. When an insect alights upon a leaf the glands or tentacles bend over and shut together and enclose it. The imprisoned insect is slowly dissolved by the secretion, and the digestive products are absorbed by the leaf. Darwin found that the secretion could not only dissolve ordinary proteid matter, but also connective tissue, cartilage, and gelatin.

Enough has now been said, perhaps, not only to indicate the mode of action of the proteolytic enzymes, but to show the important part they play in animal and plant life. We have shown how the *pepsins*, acting best in an acid solution, convert proteid bodies into soluble peptones, along with the production of transition or intermediary products known as the albumoses. Further, how the *trypsins*, acting best in an alkaline solution, likewise transform proteid substances, and produce still deeper changes in them, whereby not only peptones but crystallisable bodies also such as leucin and tyrosin are formed.

Whilst in the animal we have the double action of the peptic and tryptic enzymes as they occur respectively in the gastric and intestinal juices, the digestive action of the plant on proteid matter is mainly of a tryptic

character, and is due to enzymes, which are analogous in their action to the trypsin which occur in the digestive glands of animals. The main aim of their action in every case is the conversion of insoluble proteid matter, whether it occur as a reserve in the plant or as freshly absorbed food in the animal, into a generally diffusible or soluble body—a peptone. In this way the proteid material which is indispensable for life is furnished to the various cells of the living organism, the proteid matter being absorbed in the form of a peptone from the digestive tract of animals. What is the fate of the peptones which are thus absorbed from the digestive tract? In the blood of an animal which is in the act of digestion the peptones are either absent or only present in very small quantities. The greater part of the peptones cannot therefore pass as such into the general blood circulation, but must be once more reconverted into proteid matter at an earlier stage of its circulation. Where does this regeneration of peptone into proteid matter occur? The regeneration appears mainly to take place in the intestinal wall. The peptone disappears in the intestinal wall in its passage from the intestine to the blood. Its regeneration appears therefore to be effected, in a way we do not understand, by the cells of the intestinal wall, and in its regenerated form it is passed on by the cells to the blood.

The proteids are essential constituents in the life of a cell of an animal organism; they are absolutely necessary for the building up and the sustenance of the functions of its protoplasm. The proteolytic ferments are, therefore, amongst the main factors which minister to the life of the cell, and they do this by converting the insoluble proteid

constituents of the food into bodies—the peptones—which are soluble, are absorbed, and are transformed into proteids which can be directly assimilated by the cell protoplasm.

We have hitherto mainly considered the production of enzymes as occurring in the complex animal and vegetable organisms, and have found the process to be, at any rate in the case of the animal, a more or less specialised function of the cells of its digestive tract. How is it in the case of the simpler organisms of which many examples are to be found in the vegetable kingdom?

Amongst the fungi and moulds enzymes with marked proteolytic action have been proved to exist. If we take, for example, the ringworm organism, the fungus which causes the ringworm disease of the scalp, we find that it secretes an active enzyme which, when added to gelatin, produces a rapid and complete liquefaction of the same.¹ If we examine the unicellular organisms, *e.g.* the yeast cells, we likewise find evidence of the production of proteolytic enzymes, comparable in their action with the pancreatic trypsin. A large number of bacteria form enzymes of a proteolytic nature, as can readily be demonstrated in cultures of these organisms. A number of bacteria in the course of their development in nutrient soils which contain gelatin produce a complete liquefaction of the gelatin. If to such a culture chloroform be added so as to prevent any further growth or multiplication of the organisms, or if the organisms be destroyed by the careful application of heat, we find that the culture fluid still retains its power of liquefying fresh gelatin. The action of the

¹ A. Macfadyen, *Journ. Pathol. and Bacteriol.*, 1895, p. 176.

living cells being excluded, this result can only be due to the influence of some secretory product of the cells themselves of the nature of an unorganised ferment or enzyme. This action can likewise be demonstrated on solid blood serum on which many organisms grow well, and in some instances produce a liquefaction of the same. The bacteria can likewise attack and dissolve fibrin and egg albumin in virtue of the enzymes they produce. These enzymes appear to be analogous to the trypsins, as they act best in faintly alkaline solutions. It is interesting to note in this connection that certain disease germs produce exquisitely toxic substances which appear as secretory products of the bacterial cells, and are in their properties closely allied to the unorganised ferments or enzymes, their toxic action being exhibited in the entire absence of the living cells which produced them. We have typical examples in the case of the tetanus and the diphtheria bacillus, respectively the active agents in the production of lockjaw and of diphtheria. Thus, in the case of diphtheria it has been proved that the severe constitutional effects, the toxic symptoms affecting the centres of life, are not due to the direct action of the diphtheria organism, but to products which are produced by it at the seat of infection—the throat—and which are absorbed into the system. These products are poisonous in very minute quantities, and like the enzymes produce their effects in minimal amounts, acting likewise quite independently of the living cell which produced them, in this case the diphtheria bacillus. The grave general symptoms in diphtheria are therefore due to an intoxication of the system by an unorganised product of the diphtheria bacilli produced at the local seat of

infection, the throat, and if we cultivate the bacilli, as is quite possible to do, on a fluid nutrient soil, outside the body, such as meat broth, the cells in the course of their multiplication produce the same toxic substance, which can act independently of the living cells. This can be proved by filtering the culture, so that all the diphtheria bacilli cells are removed. The filtrate still retains the toxic properties associated with the action of the diphtheria bacillus itself, just as a filtered culture of sundry harmless bacteria contain various digestive enzymes. Just as certain cells produce specific enzymes, *e.g.* gastric cells, the pepsin, so the diphtheria bacillus produces a specific product, a toxin, to which the specific symptoms of the disease are due. And the same applies to the bacillus of tetanus or lockjaw.

This disease is mainly a local infection. The micro-organism causing the disease is located in some particular region—namely, the wound which precedes the lockjaw. At the local site of infection the micro-organisms manufacture poisons which are absorbed, circulate throughout the body, and, having a special affinity for the nerve centres, give rise to spasm of certain muscles, particularly those of the neck and jaw (hence the ‘lockjaw’), and also to convulsive seizures. If the tetanus bacillus be cultivated in broth under appropriate conditions, it is found that the broth, freed from bacilli by filtration, is intensely poisonous, acting in minute quantities, and produces spasm and convulsions—*i.e.* the specific characters of the natural disease. In the case of certain enzymes, if they be injected into an animal, its cells react by producing bodies which are capable of neutralising the action of the

enzymes. To such bodies the name of 'antiferments' has been given. Similarly, in the case of diphtheria and of tetanus, if the toxins be injected into a horse, keeping below the fatal dose, a stimulation of cells occurs, and the cells respond by giving off into the blood bodies which neutralise the respective toxins. These bodies are called *antitoxins*. If the blood of a horse containing these antitoxins be injected into a child suffering from diphtheria, it neutralises the diphtheria poison and prevents its fatal toxic effect on the system. Therefore analogies exist between the enzymes and the specific poisons of certain disease germs.

It has been suggested that in natural immunity—*i.e.* the capability of organisms to resist the invasion of germs—bodies of the nature of enzymes may be the protective agents.

If an animal be immunised or rendered resistant to the cholera or typhoid microbe, substances are formed in the blood which destroy the cholera or typhoid microbe; they digest or dissolve them up. This is termed *bacteriolysis*.

Further, if blood from a sheep be injected into a goat, the blood of the goat acquires the property of digesting or dissolving the red blood cells of the sheep. This is called *hæmolysis*, and it has been suggested that the active agents in such processes are ferments of a proteolytic nature.

It will thus be seen that the study of the proteolytic enzymes is not only of great importance as regards the digestive processes necessary to the life of animal and vegetable organisms, but that their study may likewise

throw considerable light on the nature of the specific poisons produced by bacteria in the course of a disease; whilst the phenomena to which a natural or acquired resistance to the parasitic bacteria in the animal body are due may possibly be based on factors amongst which proteolytic enzymes are to be counted.¹

¹ The digestion of bacteria by the leucocytes in the process of phagocytosis (p. 249) appears to be due to intra-cellular proteolytic enzymes, to which Metchnikoff has given the name of *cytases*.—En

LECTURE III.

The Clotting Enzymes—Curdling of Milk by Organised Ferments
—Rennet—Clotting of the Blood—Clotting of Muscle Juice—
Vegetable Jellies.

We now pass to the consideration of another group of ferments, known as the coagulating or clotting ferments or enzymes. The milk of the cow consists of an emulsion of fat—*i.e.* very finely divided fat globules, which are suspended, not dissolved, in the fluid part of the milk. The fluid part of the milk consists chiefly of proteid matter and of a sugar known as milk sugar in solution, along with certain salts. Absolutely fresh milk shows no tendency to coagulate or clot *per se*, nor does it coagulate on being boiled, at most a skin or film forms on the surface, consisting of coagulated lact-albumin of the milk. Milk has, when allowed to stand, a tendency to become sour, and this souring of the milk is due to the formation in it of acids. When this souring has reached a certain stage the milk at the ordinary room temperature coagulates spontaneously to a solid mass. The contraction of this coagulum or clot leads to the separation of the milk into a solid curd and a yellowish acid fluid. This is the result of a fermentation process, and is due to the action of various kinds of micro-organisms. Of these fermentation processes in the

milk the most important is known as the lactic-acid fermentation, which is mainly due to certain organisms termed the lactic-acid bacilli—minute rod-shaped bacteria. In the spontaneous souring and coagulation of the milk the cause is a lactic-acid formation due to the action of these lactic-acid bacilli. What is the source of the lactic acid? It is due to the decomposition of the sugar present in the milk by the bacteria in question. In addition to lactic acid, volatile acids may likewise be formed, such as acetic acid and butyric acid. The coagulation of the milk in such instances proceeds, therefore, as follows: the bacteria multiply in the kept milk, which is an excellent soil for the growth of all kinds of bacteria, and in the course of their growth and multiplication they attack the milk sugar, forming out of it lactic acid, which precipitates the proteid matter of the milk, the casein, in the form of a solid curd. We have here, therefore, an indirect action of a living or organised ferment on the milk, producing a clotting of the milk not by the direct action of the organised ferment, the bacterium, nor by the action of an enzyme secreted by it, but by an acid which it has formed through a splitting up of the milk sugar. Such a curdling or solidification of the milk may be produced by a number of other bacterial or organised ferments. And as a matter of fact the fermentable nature of the milk by micro-organisms in virtue of the sugar it contains has given rise to the preparation of various kinds of milk beverages, which, at any rate on the Continent, are frequently employed in the dieting of invalids or convalescent patients. These are familiar to us under the names of Kephir and of Koumiss, which are alcoholic

fermentations, alcohol being formed and carbonic acid evolved. That the acid fermentation of the milk with clotting is due to micro-organisms has been fully proved. If milk is sterilised by heat and the entrance of bacteria prevented, the acid fermentation and the consequent clotting do not occur; whilst if we add pure cultures of lactic-acid bacilli to sterilised and germ-free milk a souring and a clotting of the same occur, the solidification of the milk under such circumstances being due to a precipitation, through the influence of the formed acid, of the casein substance present in the milk serum or plasma. This proteid of the milk contains, like all proteids, carbon, hydrogen, nitrogen, oxygen, sulphur, and, in addition, phosphorus, and is the essential constituent of any curd formed in the course of the coagulation of milk. This curdling of the milk, due to the production of acids by bacteria, we must *not* confound with the clotting which is produced by the action of certain bodies which are of the nature of unorganised ferments, *i.e.* are of the nature of enzymes. It is these milk-clotting enzymes in which we are at present especially interested. If an extract be made from the stomach of the calf, and the extract be added to milk, a clotting of the milk quickly ensues. This fact has long been known and applied in the preparation of cheese, and it was realised that the process is different from that which occurs in the course of the lactic-acid fermentation we have just described. It was proved that this form of clotting is due to a ferment which has been termed the rennet ferment. This ferment is formed and secreted by the cells of the mucous membrane of the calf's stomach, in which it is especially abundant, and

in that of other animals. It can be obtained from the gastric cells by extraction with glycerin, and can be precipitated out of its solutions by the addition of alcohol, and still retains its active properties as in the case of other enzymes. The ferment, like the pepsin of the stomach and the trypsin of the intestine, appears to exist in the cells in the form of a pro-ferment, a mother substance or zymogen, as the generic term is. The conversion of this pro-ferment or zymogen into the active ferment or enzyme is due to the action of the free acid present in the gastric juice—the hydrochloric acid. It exerts its clotting action on the milk when it passes into the stomach, and for this action to take place the presence of *lime salts* is necessary. This is one of the most characteristic features of the casein of the milk, its faculty of clotting in the presence of a lime salt through the action of rennet. Thus, in a solution containing *no* lime salts the casein *per se* will not clot. If, however, lime salts are added clotting occurs, and in cheese thus formed a considerable amount of lime salts is present. We have here an obvious difference between the action of rennet and that of other proteolytic enzymes, *e.g.* pepsin, which converts an insoluble into a more freely soluble body—the peptone. In the case of rennet the first obvious effect is the conversion of the proteid matter, the casein, into a less soluble modification. As we have seen, the casein of the milk can be precipitated by acids formed by bacteria, and it can be precipitated if one adds an acid, *e.g.* acetic acid, and finally it can be precipitated directly by the action of the rennet enzyme. The process has a surface resemblance to the coagulation of the blood, a clot being formed

which by subsequent shrinking squeezes out a watery fluid, the whey. In the one instance we have the curd and whey, in the other the clot and serum. In the course of its separation by rennet the casein undergoes a chemical alteration, as is seen by the formation of the insoluble curd. The rennet is most abundant in the stomach of young animals, especially the calf, and it is practically a normal constituent of the gastric juice of most animals. It is likewise present in the pancreatic juice, and has been found in the stomach of fishes and birds. The ferment acts best at about the temperature of the body, and in a neutral or faintly acid or alkaline medium. The clotting of the milk does not appear to take place in the absence of lime salts. If to a pure solution of casein in water rennet be added, no clotting occurs. If, however, the casein be dissolved in lime water, the clotting at once appears. It has been suggested in explanation that the rennet chemically changes the casein into a body, named tyrein, and that the lime salt causes the latter to separate as the curd.

Whilst the rennet ferment is widely distributed amongst animals, it is also frequently met with in plants (as, *e.g.*, in *Galium verum*), and is used in this form by dairymen in Somersetshire to produce a curdling of milk preparatory to cheesemaking (? situate in the flowers), and similarly a plant containing a milk-curdling enzyme is used in cheese-making in the Italian Alps (*Pinguicula vulgaris*). The glands of the insectivorous plant not only digest proteids, they curdle milk as well. And the rennet enzyme and a proteolytic ferment are frequently to be met with associated, as in the juice of the pineapple. The fruit and seed of plants appear to be the

favourite seats of its production. A very active rennet exists in the fruit of certain South African desert plants, *e.g.* *Naras*, as well as in the seeds of certain North Indian plants, *e.g.* *Withania*. And if we extend our observation to the microscopic unicellular organisms—the bacteria—we find a number of micro-organisms which produce not only proteolytic but likewise rennet-like ferments or enzymes, *e.g.* *Bacillus mesentericus vulgaris*; whilst Conn has even succeeded in separating the rennet enzyme from the microbes. Such rennet-producing microbes are to be distinguished from bacteria which curdle milk by the formation of acids. The *rennet* clotting enzyme is thus widely distributed amongst plants and animals. At the same time it is a very difficult matter to explain the nature of the functions it fulfils, and its mode of action in relation to plant life is at present quite mysterious to us; and whilst, as regards animals, it is to be met with in their gastric juices, and may therefore perform a useful function in connection with the milk diet on which they live, the exact nature of that function we are unable to speak of definitely.

We now pass from the consideration of the clotting of milk by the rennet enzyme, whether of animal or vegetable origin, to a phenomenon which is purely animal—viz. the coagulation or clotting of the blood. The blood, although leaving the body in a perfectly fluid condition, quickly changes its properties, becoming of a viscous consistency, and finally setting into a firm jelly or clot. This change is known as the coagulation of the blood. Before considering this remarkable change more closely, we must refresh our memory as to the constitution of the blood

itself. If a drop of blood be taken directly, say, from the tip of the finger, and examined under the microscope, the first thing that strikes one is that the fluid blood is not of a homogeneous nature. It consists of two visible constituents, a nearly colourless fluid known as the plasma, in which are suspended formed bodies or particles termed the corpuscles of the blood, just as the fat globules are suspended in the fluid of milk. We have, therefore, in the blood these two essential constituents, plasma and corpuscles, *i.e.* blood cells. Blood, therefore, may be regarded as a fluid cellular tissue. If we examine the suspended bodies, the corpuscles, more closely, we find that we can divide them into two groups, each of which has marked and distinctive

characteristics. We have, first, cells called the red blood corpuscles, and cells termed the white or colourless blood corpuscles (fig. 8). The red blood cells are more numerous than the white blood cells, and as their name implies have a reddish colour, whilst the white corpuscles are



FIG. 8. — Blood highly magnified. Many red blood corpuscles and four white corpuscles or leucocytes.

unpigmented or colourless. The red cells are flattened circular discs of a diameter of $7-8\ \mu$, *i.e.* $\frac{1}{3200}$ part of an inch, and it has been estimated that 10,000,000 of these cells will lie on a space one inch square. The cells are not flat, but are bi-concave discs with rounded edges, and are not rigid but are flexible and elastic

bodies. If to a specimen of blood water be added, the colouring matter of the red blood cells passes into the surrounding fluid, the cells becoming quite pale or colourless. The spongy colourless framework of the cell is known as the *stroma*; the pigment to which it owes its colour and which can be removed is known as *hæmoglobin*. This consists of an iron-containing body, hæmatin, in combination with a proteid substance. This colouring matter or hæmoglobin constitutes about 90 per cent. of the whole cell. It can unite with oxygen, and is the great carrier of oxygen to the tissues of the body. In man the red blood cells contain no trace of a formed body or nucleus.

The white cells, also termed leucocytes, are larger than the red—measuring $10\ \mu$, or about $\frac{1}{2500}$ of an inch—and are irregular in shape. They are free wandering cells or units of life, and most of them exhibit spontaneous amœboid movements. They consist of finely granular protoplasm with a contained body, a nucleus. Only 10–12 per cent. of these cells is solid matter; the remainder is mainly water. The essential constituents of the solid matter are proteid bodies. These cells can ingest solid particles from the blood and absorb them into their substance, and may likewise discharge substances into the blood, and in this way it is probable that they have something to do with the production of the clotting of the blood when it is shed. We must mention a third class of particles that exist in the blood, the blood platelets, which are extremely minute, and about which little or nothing is known. There are about two or three white blood cells for every 1,000 red blood cells in a

specimen of blood. The average blood consists of about two-thirds plasma and one-third of corpuscles. When we consider the chemistry of the blood we find it to be a slightly alkaline fluid consisting of watery, solid, and gaseous matters. Of the gases the most important is oxygen. As regards the plasma of the blood, it contains about 90 per cent. of water and 10 per cent. of solids, mainly proteids, which, as we saw, are built up out of carbon, hydrogen, nitrogen, oxygen, and sulphur, and are nitrogenous bodies. It is essential that we should know of the important proteid constituents of the blood plasma. They are three in number. If we add to blood plasma 15 per cent. of ordinary salt a precipitate occurs of the first of the three substances—*fibrinogen* (coagulates at 56° C.). If the plasma from which the fibrinogen has separated be saturated with as much salt as it can take up, a further precipitate occurs of a body called *para-globulin* (coagulates at 75° C.). There still remains behind in the blood the third substance, known as serum albumin—very like the albumin in the white of an egg. Though coagulating like the globulin at 75° C. it is not precipitated by salt from its solutions. We have now learned the main constituents of the blood. It consists essentially of a fluid having suspended in it cells, some of which are pigmented non-nucleated cells, the red blood cells; others much fewer in number are nucleated, free-living, wandering or amœboid cells and are colourless—the white blood cells. The fluid of the blood, the plasma or blood serum, contains gases of which the most important is oxygen, a large percentage of water, and, most important of all, three proteid substances, termed fibrinogen, serum globulin

and serum albumin. We are now in a position to consider the clotting phenomenon as it occurs in this fluid blood. If the blood shed from an animal be collected in a vessel it is at first quite fluid, but a change quickly appears, the blood changes from a fluid to a more solid or jelly-like consistence, and a firm clot is formed, just as we noticed in the case of milk on the addition of the rennet ferment. The appearance and consistence of the blood may be compared with that of red-currant jelly. It does not, however, remain permanently in this condition, a shrinkage of the jelly-like substance occurs, and a fluid oozes out of it, so that we have a separation of the whole mass into two parts—a central semi-solid portion, the clot, and an outer fluid portion, the serum. As in milk we had a curd and whey, so in the case of the blood we have a *clot* and *serum*. The clot has a reddish colour; the serum is clear and of a yellowish colour. The cause of the red appearance of the clot is that it contains within it the red cells of the blood. And if the clot be examined microscopically, we can detect the red blood cells entangled in the meshwork of a fibrous-looking substance, which forms the skeleton or framework of the clot. This fibrous-looking material is termed *fibrin*. The clot, therefore, consists of *fibrin* derived from the blood, along with the *blood cells* entangled in its meshes. The clotting has therefore removed from the blood, as a whole, its cellular-formed elements, along with a certain unformed chemical constituent of its fluid part—viz. the fibrin. The clot consists of *fibrin* and *blood cells*; the squeezed-out fluid or serum consists of the original blood fluid or plasma, *minus* the fibrin or equivalent substance which it contained in

its normal condition in the body. The clot may be uniformly red, but if the clotting takes place slowly, the red blood cells have time to sediment or to sink towards the bottom layers of the fluid before the fibrin appears. The upper layer of the clot is therefore of a pale colour, and has been termed the buffy coat ; it is best seen in horse's blood, which clots slowly. If the blood is whipped whilst clotting is proceeding, the fibrin adheres to the twigs. The fibrin when washed from the last traces of the red blood cells appears as a white, stringy, elastic substance. This spontaneous clotting of the blood may be affected or modified in various ways. A temperature rising up to about blood heat hastens the clotting, whilst it is greatly delayed if the blood be kept at a cool temperature in an ice-chest. If salts be added, such as common salt, the clotting does not occur. The clotting is promoted by the blood coming in contact with dead matter, as, *e.g.*, the sides of the vessel in which it is received ; whilst if a wire be introduced into the vein of an animal, fibrin or clot is deposited on it. If a portion of a vein be ligatured at each end, so that the blood is cut off from the general circulation and is enclosed in a living chamber formed by the walls of the vein, the blood remains fluid for a very long time, being in contact with living matter, although blood removed from the enclosed portion of the vein clots immediately on removal. The following is a well-known experiment, and shows that the clotting is due to a modification of certain substances present in the normal fluid blood. If fresh unclotted blood is saturated with salt a precipitate occurs of a body, called *plasmine*, which, however, can readily be

redissolved in water ; in this respect it differs from fibrin, the formed fibrin in the clot being an insoluble substance. This solution of plasmin, however, if allowed to stand, becomes converted into an insoluble or jelly-like substance, *i.e.* a clot consisting of genuine fibrin. In the process of clotting of the blood the fibrin, therefore, must be derived from this substance—*plasmin* ; the soluble plasmin is converted into the insoluble fibrin. How does this remarkable change take place ? The subject has not yet been by any means definitely settled, but there are certain well-known observations which tend to throw some light on the process. In serous cavities of the body—*e.g.* heart pericardium—there is a clear fluid which consists of blood plasma without the red blood cells. This fluid usually does not clot spontaneously. If, however, a small quantity of already clotted blood be added to it, clotting ensues. The serum contains a substance termed ‘fibrinogen,’ which is the antecedent of the fibrin which forms the basis of the clot. By adding already clotted blood to the serum containing this fibrinogen, a substance must have been added which did not exist in the serum inasmuch as it remained unclotted *per se*. The fibrinogen is the antecedent of fibrin in each case, and therefore some agent must have existed in the one case which did not exist in the other to produce the transformation into fibrin—in other words, into a clot. The substance which produces this change has been termed the *fibrin ferment*, which appears in the blood when it has been removed from the body. As to its origin we have no exact data, although it has been supposed that it may originate either from the white blood cells or the blood platelets. The theory

propounded is that the fibrin is formed by the action of the fibrin ferment on the fibrinogen.

The coagulation of the human blood occurs in about seven to eight minutes. The rationale of the clotting has long been a crux to physiologists. A view commonly held was that it is due to the removal of the blood from the influence of the living, uninjured walls of the blood-vessels. We have in favour of this the experiment of Lister and others, the blood remaining fluid in a ligatured vein. What is the nature of this influence of the walls of the blood-vessel? Blood remains fluid if drawn through an oiled cannula into an oiled vessel. And if it be whipped with an oiled glass rod it does not clot, but does so if the rod be *unoiled*. Freund therefore supposes that clotting is *started* by an adhesion between the blood elements and a foreign body, and in this way the process is initiated. The process does not occur in normal circulating blood, owing to absence of this contact or adhesion. As to the mechanism of the process thus started opinions differ. Some suppose that by a decomposition or breaking down of the white blood cells, the active agent in clotting passes into the blood plasma. Others suppose that this occurs without a decomposition of the white blood cells—the active substance diffusing out of them—whilst others believe that the process originates in the blood platelets. The various opinions agree in this, that the active agent is produced from some one of the formed elements of the blood. Schmidt supposes that the leucocytes and cells of the tissues generally contain *two* groups of substances, of which the one group hinders and the other promotes clotting of the blood. During life the

preventive group of agents is predominant, whilst outside the system, or on contact with a foreign body, the cellular agents that favour clotting take the upper hand.

There is one factor which, whilst we do not know how it acts, is an essential one, and that is the presence of *lime salts*. As I have indicated, the clotting of the blood is generally regarded as due to the action of a ferment—named the fibrin ferment—which, whilst producing the clotting, remains itself unchanged in the process. By its action, out of soluble fibrinogen the insoluble fibrin is produced, just as in the case of the rennet ferment we have the production of the solid curd from the casein of liquid milk. To this hypothetical fibrin ferment the name of *thrombin* or *thrombase* has been applied. This thrombin, which may be a nucleo-proteid, can be obtained from the fibrin or from the serum which is pressed out from the clot, and its formation appears to take place at the moment of coagulation, and it does not therefore exist as such in the normal blood. It is supposed that this *thrombin* exists in the living body in the form of a mother substance, a pro-ferment or zymogen—termed *prothrombin*—and that the lime salts mentioned as being necessary to the clotting process are concerned in the conversion of the zymogen or pro-ferment into the active ferment, the *thrombin*. On this hypothesis the clotting of the blood may be represented as follows: 1, calcium salts + prothrombin = fibrin ferment; 2, fibrin ferment + fibrinogen = fibrin. This will, perhaps, suffice for our discussion of the blood-clotting ferment. You will see that there are many points connected with the nature of the active agents and the nature of the process that are

obscure, and that the explanations in many instances given are of a hypothetical nature. The active agent, it is generally supposed, is of the nature of an enzyme. In the rigidity of the muscles which occurs after death—*i.e.* during *rigor mortis*—a formation of a substance named *myosin* occurs in the muscle juices. If the muscle juice be extracted, the juice on standing becomes gelatinous—it forms a clot. The myosin of the muscle-judge clot is comparable with the fibrin of the blood clot. This coagulation of the muscle juice with the formation of myosin is due to the action of an enzyme—which can be extracted from the muscle—and can produce a clotting of fresh muscle juice. This enzyme cannot clot blood, nor can the blood ferment clot muscle juice; these ferments are, therefore, distinct from one another. It would appear therefore that the *post-mortem* muscle rigidity is due to the action of a soluble ferment or enzyme, termed the myosin ferment, which may be ranged with the clotting ferments or enzymes. We have now traced the existence of three ferment-like bodies or enzymes which produce a clotting of certain proteid substances—*viz.* the rennet, which curdles the casein of the milk; the fibrin ferment or thrombin, which clots the fibrinogen of the blood; and the myosin ferment, which clots certain proteids of the muscle juice with the formation of myosin. We will conclude our consideration of the clotting enzymes with a short reference to a clotting ferment, not of animal but of vegetable origin, to which the name of *pectase* has been given. I will follow the description of it as given by Reynolds Green. The formation of the vegetable jellies as prepared from ripe fruits is due to the action of an

enzyme upon a substance which can be extracted from the cell sap or cell walls of many vegetable tissues. This substance is called *pectine*, and by the action of the enzyme pectase it can be converted into two gelatinous bodies, pectosic and pectic acids. The enzyme Fremy found to exist in two forms, either in solution in the neutral sap of roots such as the carrot or beet, or in an insoluble state as in the juice of acid fruits. Such juices added to pectine solutions produced a rapid gelatinisation of the pectine. The enzyme itself can be extracted from the juice of young carrots by precipitation with alcohol. The results of its action, the pectic acids, are uncrystallisable bodies, and are closely related to the carbohydrates, containing carbon, hydrogen, and oxygen. The active enzyme as prepared from the juice of carrots gelatinises solutions of pectine when added to them. The jelly appears to be pectic acid united with lime to form a compound body, viz. a pectate of calcium. And it is an interesting fact that here likewise a calcium salt is necessary for the formation of the clot, just as is the case in the curdling of milk by the rennet ferment. Bertrand and Mallevre found that the enzyme, pectase, was not confined to pulpy plants only, but that it was widely distributed in the vegetable kingdom—for example, in the growing foliage leaves of green plants (*e.g.* Lucerne). The wide distribution of pectase, along with the universal presence of pectic bodies in conjunction with cellulose in the cell walls of plants, has suggested that it is intimately connected with the changes undergone by the cell wall during the life of the cell. As a matter of fact, the vegetable cell wall does not consist of *pure*

cellulose ; it contains other substances of the nature of pectines and of pectic acid, as in the so-called inter-cellular substance. It is supposed that the cell wall consists of a middle layer of calcium pectate, the outer and inner layers consisting of cellulose and pectose ; and where cell growth is most vigorous, the more plentiful is the enzyme, the pectase. The function of pectase is not very clear. It may assist in swelling up a cell wall previous to its solution, and it is recognised by its power of forming vegetable jelly from the pectic substances of the cell wall, and this jelly appears to be a compound of pectic acid and calcium.

We have now proceeded so far in our consideration of the soluble ferments or enzymes that we have traced in broad outline the salient facts that are known with regard to two important groups which exercise a specific action on proteid or nitrogenous matter. We dealt first with the proteolytic enzymes, which convert proteid matter into soluble or assimilable bodies, the peptones, which eventually are taken up by the cells for purposes of nutrition. Of these the most typical examples were pepsin, as present in the gastric juice, and trypsin, as present in the pancreatic juice in the intestine, and they were, as we saw, essential factors in the processes of digestion. Such enzymes were produced, not only by animal but likewise by vegetable cells, and their presence and share in the support of the processes of life were of such a universal character that they were to be detected even in the humblest forms of life known to us—viz. the unicellular free-living vegetable cells, such as the bacteria. We have dealt secondly with a group of ferments characterised by

a different action on proteids, inasmuch as they convert the proteids not into a more soluble modification, as in the case of the first group, but into a less soluble modification. These were the clotting ferments or enzymes. We referred to the rennet ferment or chymosin and its action on milk, which consists in a conversion of the casein into a clot or curd, and a separation of the fluid milk as a consequence into a solid curd and a watery whey ; also to the fibrin ferment or thrombin, which converts the fibrinogen of the blood into a solid substance—the fibrin—with a separation likewise of the fluid blood into a solid clot and a fluid serum or plasma. And we touched upon the myosin ferment, which gelatinises proteid matter present in the muscle juice, this clotting resulting in the *rigor mortis* of the muscles. And, finally, we referred to the plant enzyme, the pectase, which is responsible for the formation of plant jellies, as in pulpy plant tissues.

LECTURE IV.

Enzymes acting on Starches and Sugars—Diastase—Tonkin Yeast—Saliva—Saccharification of Starch—Functions of the Liver—Inulase—Digestion of Cellulose—Cytases—Sugar-splitting Enzymes—Invertase—Glucoside-splitting Enzymes—Fat-splitting Enzymes or Lipases.

We have seen that the main elements of the food material of a living organism consist of nitrogenous substances, the proteids, and of non-nitrogenous substances, the carbohydrates and the fats; and we may now consider the changes undergone by the starchy food elements, the carbohydrates, as a result of the action of certain soluble ferments or enzymes, produced just as pepsin and rennet are by the cells of plants and animals in the course of their life processes.

We will begin with the *sugar-forming* or *saccharifying enzymes*. Under the term saccharifying enzymes we understand those which exercise their peculiar action on the carbohydrates, and convert the more complex carbohydrates into simpler substances, for example, starch into sugar, *i.e.* a saccharification of the starchy matter occurs. Such saccharifying enzymes are widely distributed in the animal and the vegetable world; and their function is an important one in the economy of the living organism, inasmuch as they convert unassimilable starchy matters into simpler and soluble modifications—the sugars, which

can be used for the vital processes of the cell. They perform the same useful function in connection with the carbohydrates of the food that is carried out on the proteids by the proteolytic ferments or enzymes, viz. an act of solution or digestion—a conversion into a body that can be assimilated by the cell for the purposes of its vital activity. The generic term for such starch-splitting enzymes is *diastase*. These diastatic ferments are very widely distributed. Their existence was first detected in the germinating barley grains, which were found to contain a substance which, when extracted from the grain by water, was capable of converting starch into sugar. Its presence was demonstrated not only in germinating barley but likewise in the seeds of other plants, *e.g.* in oats, maize, and rice. Diastatic enzymes have likewise been found in many vegetable tissues and saps—in fact, in starch-containing plant saps generally. This led to the general conclusion that where starch is found in a plant a diastase is likewise present, and that the function of the diastase is to carry out the starch-dissolving properties of the plant which are so essential to its life processes. In fact, the solution of the insoluble starch in the plant is due entirely to the action of diastatic enzymes. This generalisation, as regards the saccharifying of starchy matter being wholly and always due to a soluble ferment or enzyme, has not been accepted by some observers; at any rate, two kinds of diastase appear to exist in the plant. The first is the most universal form, and appears to be engaged in the transport of starch from one part of the plant to another, and is to be found in the vegetative organs. The second form of

diastase is mainly met with in germinating seeds. These have respectively been termed the diastase of *translocation* and the diastase of *secretion*. We shall have occasion to refer to them more fully at a subsequent stage. Diastase is not a substance occurring diffusely in the plant organism, but is a genuine secretion product of its cells, *i.e.* a soluble unorganised ferment or enzyme. It is interesting to note that, just as in the case of the proteolytic ferments, the diastase appears to exist in the higher plants in the form of a pro-ferment or zymogen. Such saccharifying ferments are likewise produced by the lower plants, such as the algæ and fungi. The fungi or moulds, *i.e.* the class of organisms with which we are so well acquainted as growing on jellies or in cheese, have considerable practical interest as producers of diastatic enzymes. Certain kinds of moulds, in virtue of the diastatic enzymes they produce, can convert starch into a fermentable sugar, *i.e.* into a sugar which, under the influence of *yeast*, can undergo the alcoholic fermentation. And this fact has been utilised in Asiatic countries for the preparation of alcoholic beverages, especially those made from rice; one of the best known is the Koji 'yeast' used in the preparation of the Japanese liquor, *saké*, from rice. It contains a mould, the *Aspergillus orizæ*, which produces a diastatic ferment, the Taka diastase, which converts the starch of the rice grains into sugar—that is, into a form in which it can be attacked by the yeast cells and an alcoholic fermentation set up. Similarly, we have the Tonkin yeast, as investigated by Calmette, which contains a mould, rich in diastase, along with an alcoholic yeast. The diastatic ferment of the mould furnishes

fermentable sugar to the yeast. We have in the Japanese and the Tonkin yeasts interesting examples of a symbiosis between a diastase-producing mould and an alcohol-forming yeast. Amongst the unicellular vegetable organisms—the bacteria—the property of converting starch into a soluble sugar is of wide occurrence, and here likewise the action of the bacterial cells appears to be due to the activity of diastatic enzymes which they secrete. Diastatic ferments likewise have an important function in the digestive processes of animals, inasmuch as they convert insoluble starchy constituents of the food into soluble sugars, which can be used by the various cells of the organism for purposes of nutrition. In animals such diastatic enzymes are to be found, especially in the saliva of the mouth, in the pancreas, in the intestinal juices, and in the liver. In all these instances they are, as in the plant, genuine enzymes. The saliva of the mouth, added to starch paste, converts the starch into a soluble sugar, in the same way as occurs through the action of a vegetable diastase. A similar diastase is produced by the pancreatic gland and is excreted into the intestine, and produces similar effects on starchy matters. The liver contains a starchy carbohydrate, which may be termed animal starch. To this starch-like substance the name *glycogen* has been applied, and Claude Bernard has shown that glycogen after death, and also during life, is readily converted into sugar. It seems, therefore, a probable supposition that the conversion of the animal starch or glycogen into sugar is due, as in the case of vegetable starch, to the action of a diastatic *enzyme*. This is one of the most important questions in physiology: the production

and the nature of glycogen and the nature of its decomposition products. The transformation of glycogen has every appearance of being a ferment process. Diastatic ferments have been detected in most of the organs of the body, as well as in the blood. And one may mention that the disease *diabetes* is in its origin and course closely associated with the action of diastatic or saccharifying ferments. The general function of a diastase, wherever met with in plant or animal, is to transform starch or glycogen into sugar. The starch is an important reserve material for purposes of nutrition in the case of the plant, and it is stored by the seed or the plant for this purpose. And when the plant wishes to use this reserve food or starch, the first act on its part is the conversion of the starch into a soluble and transportable form, *i.e.* into a sugar. And in a similar fashion the liver stores up animal starch or glycogen as a reserve, which can, as required, be converted into a soluble sugar, and distributed to the organs or tissues of the body which may need it. The physiological importance of starchy matter in the vegetable or animal economy, as well as the important function of the starch-transforming enzymes or diastases, is readily apparent. And we will now consider these saccharifying enzymes more closely, commencing with the vegetable *diastases*, which we will deal with as briefly as possible. The most familiar example, as I have said, and the one which has been most closely studied, is the malt diastase, which was discovered and described in 1833. It has the properties of a genuine enzyme, and acts independently of the cells by which it is produced, just as in the case of pepsin or of the rennet ferment, whilst, like other enzymes, a high

temperature may paralyse or completely destroy its action. Its action consists in the conversion of starchy matter into sugar. The two varieties of the vegetable diastase are known as the diastase of translocation and the diastase of secretion. The diastase of translocation, as its name implies, is concerned in the transport of starch from one part of the plant to another, and is therefore commonly met with in the vegetative or growing parts of a plant. Its essential function is the conversion of starchy reserve material into a soluble assimilable sugar, which can be utilised for purposes of nutrition by the plant cells as a whole. The diastase of secretion is to be particularly met with in germinating seeds—*e.g.* in the grains of grasses. Its formation takes place in the process of the germination of the grain. Whatever the nature of the vegetable diastase, its function is to transform starch into sugar—*i.e.* to place at the disposal of the plant, whether it be the germinating seed or the growing leaf, the store of starch it has laid up for purposes of nutrition. Such saccharifying enzymes are produced, not only by the higher plants, but likewise by the lowest forms of vegetable life—the bacteria. Thus the bacillus of cholera asiatica can liquefy starch paste, and can convert the dissolved starch paste into sugar; whilst, as we have seen, a number of moulds have similar properties, and their action in the course of certain fermentations is of great importance—*e.g.* in the case of the Japanese and Tonkin yeasts, which convert the starch of the rice grain into a fermentable sugar, which by the action of various kinds of yeasts is split up into alcohol and carbonic acid, and in this way an alcoholic beverage is obtained. Like the other enzymes, the diastase has not

been isolated in a pure form, the extracts of it from the vegetable cells containing other substances besides the active agent or ferment; its action, however, on starch can be readily demonstrated in the test-tube, as in the case of other enzymes. The process of the conversion of starch into sugar by the action of diastase is a complicated one; and it is not my intention to enter into a discussion of the nature of the complex chemical processes that take place, but only touch upon them in the broadest outline. For the study of the action of diastase on starch, a starch paste is prepared by boiling starch in water. In this way a thin starch paste is prepared; to this the diastase as obtained from the plant or any other source is added, and the changes that occur can then be noted. The saccharifying action of the diastase on the starch is not an immediate but a gradual one. As in the case of the conversion of insoluble proteids into soluble peptones by the action of pepsins intermediate products are formed, so in the case of the conversion of insoluble starches into soluble sugars by the diastases analogous modifications of the carbohydrates occur. Thus, if to a starch paste a few drops of iodine are added, a deep blue colour is obtained. This is a typical reaction of pure chemically unchanged starchy matter, and, as a matter of fact, this test is one which is applied for the detection of pure starchy matter in vegetable and animal tissues, as well as in animal and vegetable cells. We know that we have a suspension of pure starch in the test-tube by the bluing that occurs on the addition of iodine. This starch paste is opalescent from the presence of the suspended starch—it is not really in solution. The starch itself exists in the plant in the form

of distinct grains or granules, being insoluble in the watery cell sap (fig. 9). These starch grains exhibit various forms in different plants; and this enables one, with the aid of the microscope, to determine the source of origin



FIG. 9.—Cell of a pea seed containing starch grains. (After Sachs.)

of any given starch grain. This microscopic examination is largely employed by the food analyst to detect adulterations of foods with starchy matter. The analyst is not only able to say that an adulteration has been made with starch, but likewise to determine the source of the starchy material used, as the starch grains show peculiar markings or stratification in their structure, which vary in form and size in different plants.

On heating these grains with water they swell up and burst, and in this way we obtain our starch paste—it is a suspension of the starch in water, and not a true solution. If to this a diastase is added certain visible changes occur. The fluid loses its cloudiness and becomes clearer and transparent. The starch has gone into solution; it has been converted, first of all, by the diastase into a soluble starch, which, however, is still chemically unaltered, as it continues to give the starch reaction with iodine. This soluble starch, however, speedily undergoes distinct chemical change; as evidence of this, a sample taken a little later gives a purple colour and no longer a blue tint on the addition of iodine. At a still later stage the reaction with iodine is

no longer purple, but of a deep red brown colour. Finally it is found that the fluid ceases to give any reaction with iodine at all—the starch has undergone a complete change into some other substance. If during this process of digestion of the starch by the diastase the fluid is tested from time to time by the addition of a reagent containing cupric oxide, it is found that it has acquired a property which it did not formerly possess, viz. that of reducing the cupric oxide to cuprous oxide, a substance which, on heating the fluid, becomes deposited as a distinct yellowish or reddish precipitate. This power increases, and is one that is not possessed either by starch or diastase, and is due to the appearance and increasing formation of sugar, for which cupric oxide is a delicate and sure test. You will see, therefore, that we have had a series of changes set up in the starch. We had, first, the soluble starch formed, and still bluing with iodine; then the formation of substances which gave, not a blue, but a purple or red brown colour with iodine; and, finally, a disappearance of any reaction with iodine, and the appearance of a reducing action on cupric oxide, representing the transformation into sugar. To the intermediate products the name of dextrins has been given, and they are known respectively as erythro- and achroo-dextrin—the end product is sugar. The stages are then: 1, starch; 2, soluble starch; 3, the erythro-dextrin, staining red with iodine; 4, achroo-dextrin, not staining with iodine; 5, sugar. The starch molecule appears to break down by a series of hydrations or additions of the elements of water to its molecule, the result being a sugar (maltose) with a dextrin of less molecular weight. The chemical

questions are of too complicated and difficult a nature to detain us at this moment. We have traced in broad outline the process of saccharification of the starch by the action of diastase; and this function is exercised in the plant for the special purpose of converting its starchy food reserves into soluble and assimilable forms, *i.e.* sugars which can be used for purposes of nutrition or constructive processes on the part of the cell. Diastase shares the cardinal properties of other enzymes; it requires a certain temperature for its action, and it is not used up or exhausted in the course of its activity. If we turn to the animal kingdom we likewise find that such saccharifying or diastatic enzymes are of wide occurrence. In man they are to be met with in the saliva, where the starchy elements of the food at once come into contact with a diastatic enzyme, this particular enzyme as present in the saliva being known as *ptyalin*. And in the intestine active diastatic enzymes are secreted which attack, saccharify, and render absorbable the starchy elements of the food. It is in the intestine, and not in the mouth, that the active saccharification of the starchy food really takes place. One of the most important organs in the body, the liver, stores up a large quantity of the sugar absorbed by it from the blood in the form of a starch-like substance or glycogen, as it has been termed. Indeed, one may term glycogen an animal starch. This reserve of glycogen is converted into sugar as it is wanted, and passes into the blood for utilisation in the general requirements of the organism. This glycogen function or production of animal starch is of such importance in the economy of the body that we may devote a short time to its consideration.

The chemical processes which occur in the liver form one of the most difficult problems in the physiology of the body. That the blood has a constant composition is due to the action of two organs—the kidney and the liver. The kidney removes from the blood all foreign substances as well as the waste products of the animal economy; what passes out of the blood passes away by the kidney. On the other hand, the liver controls everything that enters the blood; all the blood which comes from the digestive tract laden with absorbed food substances must first pass through the liver before it is pumped to the different organs and tissues by the heart. The liver is therefore placed as a sentinel in the course of the blood stream which passes from the digestive tract to the heart. It is due to the liver that the blood is not overladen with sugar, whilst at the same time it ensures a proper supply of this important foodstuff to the blood and the tissues. It ensures the conversion of poisonous substances, such as ammonia, into harmless compounds such as urea and uric acid; whilst toxic putrefactive products, which may arise from the food in the intestine through the action of bacteria and become absorbed into the blood, are converted into harmless modifications. Many poisons, such as arsenic, are likewise held back by the liver. Besides these controlling functions the liver forms an important secretion, the bile, which is discharged into the intestine, and which, amongst other things, aids in the absorption of fat by the blood. The function of the liver with which we are especially concerned is the formation of glycogen or animal starch. A portion of the sugar which is absorbed by the blood from the intestine is deposited in the liver

as glycogen. Glycogen has a similar *rôle* in the metabolism of animals to that of starch in the metabolism of plants, inasmuch as it is a reserve of starchy matter which is formed by the animal to be drawn upon as required. The storehouse is the liver, and, as it has been put, 'the loss to the blood is temporary—no more real loss than when a man deposits at his banker's some money which he has received until he has need to spend it.' The largest amount of glycogen found in the liver of animals is about 10 per cent. It is not only stored up in the liver, but likewise in the muscles, in which it may reach 1 per cent., and it appears to form the fuel for the work of the muscles. In the course of excessive physical exertion it has been noted that the glycogen disappears from the liver and the muscles; it has been used up. It may be found in small quantities in most tissues of the animal body, and is an important constituent of such tissues which are undergoing a rapid growth or cell multiplication. It has been found in numerous fungi, and is an important constituent of yeast cells. Starvation lessens and a meal increases the amount of glycogen in the liver. The glycogen when obtained from the liver is an amorphous powder, which forms an opalescent solution in water. It is fermentable, like the vegetable starches, by the action of diastatic enzymes, which convert it into sugars (maltose or glucose), and intermediate substances likewise appear to arise—viz. dextrins. What is the origin of this animal starch or glycogen in the animal body? Its main sources are *sugars*, along with the dextrins and starches, and of these the chief appears to be *grape sugar*. It may be mentioned that a portion of the glycogen may

possibly be formed from proteid material as well. The reserve glycogen is transported from one organ to another, probably in the form of grape sugar, in the blood stream. The blood and lymph contain a diastatic enzyme which can convert glycogen into sugar. It is therefore probable that the glycogen is not conveyed as such by the blood to the organs, but is formed out of the sugar when it reaches the tissues or cells. Indeed, the formation of glycogen appears to be a general function of cells, occurring not only in the animal but in the simple vegetable cell. The formation of sugar from it appears to be due in all cases to the action of a diastatic enzyme; it is a ferment action.

I think I have now said enough to indicate the importance of starchy as well as of proteid material as a food both for the vegetable and for the animal organism. The essential preliminary to its absorption in the course of digestion is its conversion into a soluble body, *i.e.* a diffusible sugar, and this is effected by diastatic enzymes. This sugar may be at once used for the purposes of nutrition, or it may be reconverted into a starchy material and stored as such in the cells until it is required for the special purposes of the organism. This storage in the animal takes place, *e.g.*, in the liver, where it is deposited as animal starch or glycogen. When required it is once more converted into a transportable form—into a sugar—and this action likewise appears to be due to diastatic enzymes produced by the cells of the body. In this way the carbohydrates or starchy matters of the food are manipulated as required in the processes of life. Whilst starch and glycogen are the main carbohydrate reserve

materials in the plant and animal world, the vegetable kingdom possesses another carbohydrate reserve in the shape of inulin, which usually exists in a condition of solution in the sap of certain plant cells (dahlia, &c.). In the course of the growth of the plant the conversion of this inulin into a sugar (fructose) takes place under the influence of a soluble ferment or enzyme, which has been termed *inulase*. Whilst, however, acting on inulin the enzyme has no action on starch, which it is unable to saccharify; it is specific.

In *cellulose* we have a carbohydrate body, or rather carbohydrate bodies, which form the main constituent of the walls of plant cells, and of which we have familiar examples in cotton-wool and in filter-paper. Cellulose is insoluble in cold or hot water, and is resistant to the action of dilute acids or alkalis. Hence its constant use in the chemical laboratory in the form of filter-paper on account of its indifference to the action of the ordinary chemical reagents; it is one of the most resistant substances to chemical change with which we are acquainted. Cellulose can by prolonged boiling with sulphuric acid be converted, like other carbohydrates, into a form of sugar (glucose), but is resistant to the action of all the ordinary enzymes, and is left untouched by them. It is therefore a remarkable fact that, despite this resistance, the enormous amount of cellulose occurring in the dead *débris* of plants in Nature does actually become broken up and resolved into simpler constituents, many of them of a gaseous nature, the most typical being marsh gas, which is formed where vegetable matter is undergoing decay. Indeed, the redistribution of cellulose is one of the longest processes occurring in the

economy of Nature. This decay and break up is a fermentative process, and is due mainly to the action of micro-organisms, which decompose it into organic acids and gaseous products such as marsh gas. Indeed, artificial cultures of certain bacteria acting at high temperatures can produce a remarkably quick and complete resolution of pure cellulose in the test-tube, a resolution more complete than can be accomplished by any of our chemical agents—not merely a transformation, but a splitting up and entire destruction of the substance as a chemical entity. It is an interesting question, What is the fate of the cellulose which is consumed by man along with his vegetable food? For a long time cellulose was looked upon as entirely indigestible, until it was found that in the case of the lower animals about 60–70 per cent. of the cellulose absorbed with their food disappeared in their digestive tract, whilst a certain portion disappears likewise in the human digestive tract. At the same time none of the ordinary digestive enzymes when tested on cellulose outside the body produces any digestive effect on it. Still, as we have said, a portion of it disappears in the digestive tract. Some suppose that the epithelial cells of the intestine may exert a fermentative and dissolving action on it. Others suppose that the cellulose is not utilised at all as a food by the body, but that its decomposition in the digestive tract is due to the action of bacteria, with a resultant production of carbonic acid and marsh gas. There is no doubt that in the digestive tract of man, and especially of herbivorous animals, a gaseous decomposition of cellulose occurs by the action of bacteria. It is, however, still

doubtful if all the cellulose that disappears has been disposed of by purely bacterial agents. Whilst the bacterial digestion of cellulose is an undoubted fact, the action on it of any human digestive enzyme has not yet been proved. On the other hand, it has been stated that the digestive fluid found in the intestine of the earthworm digests cellulose. Some state that they have found a cellulose-dissolving enzyme in the intestine of rabbits, as well as in the case of other animals. Fish are said to possess such an enzyme—*e.g.* the carp—as well as various forms of snails. The subject is one which well deserves further investigation, viz. as regards the evidence for the secretion of cellulose-dissolving enzymes by animal cells or organs. We have more definite information on this point in the case of vegetable cells. Besides the reserve starch we have already alluded to, the seeds of many plants contain as well considerable quantities of carbohydrate reserve food material in the shape of cellulose—reserve cellulose, as it has been termed, or similar cell wall forming substances. This cell wall reserve substance may be so great in quantity as almost to obliterate the cell cavity, *e.g.* in palms. During the germinative process it has been noted that large quantities of this reserve cellulose dissolve simultaneously with the reserve starch of the cells; the thick cell walls dissolve and disappear, and undergo an analogous transformation to that of the starch, inasmuch as the cellulose yields some form of sugar. This, as in the case of the starch, points to the action of some kind of ferment or enzyme. It may be pointed out that the plant cell wall contains two groups of substances—viz. the cellulose bodies and the group of pectose substances. A

cell wall does not therefore entirely consist of cellulose, and the study of the digestive changes occurring in the plant cell wall is consequently not an easy matter. The dissolving action on cellulose at any rate appears to be brought about by the action of a ferment or enzyme, to which the name *cytase* has been applied. It was discovered by De Bary in the course of a most interesting series of investigations carried out by him on a peculiar fungus which attacked the roots of carrots and turnips, forming a dense thread-like growth in the interior of the roots, which as a result became completely softened. Microscopical examination showed that the fungus interposed itself between the plant cells, and converted the cell walls into a soft mucilaginous substance, along with a swelling of the cells. The sap of the carrot expressed after the fungus had attacked it possessed the same property of dissolving cell walls. The action was therefore due to something formed by the fungus, and proved to be a soluble ferment or enzyme, the *cytase*. It is a secretion of the protoplasm of the fungus, and like other enzymes is destroyed by boiling. By its action in softening cell walls, it may permit the growing filaments to bore into and enter the protoplasm of a cell, in this way overcoming the otherwise resistant barrier of the cell wall. Cytase therefore plays an important part in the development and nutrition of certain fungi. Cytase exists likewise in the higher plants, and has been especially studied by Horace Brown and Morris in connection with the germination of barley and other cereals in which a dissolution of the cell walls occurs. The enzyme itself, the cytase, can be extracted from the germinated barley. Its secretion appears to be

dependent on the necessity for obtaining nutriment felt by the embryo plant in the course of development. The exact nature of the products resulting from the action of cytase has not yet been fully studied. The cellulose in the plant, however, like the starch, appears to be converted into some form of soluble sugar (? glucose). In the parasitic plants or fungi which destroy wood, it is probable that a cytase or cellulose-dissolving enzyme is produced.

We have proceeded so far in our study of the soluble enzymes that we now have considered the proteolytic enzymes which act on proteid matter, such as pepsin and trypsin, the clotting enzymes which convert proteid matter into a less soluble modification, such as rennet and its action on milk, and the fibrin ferment and its action on blood; and we proceeded to the study of the action of certain soluble ferments on the second great group of foodstuffs, the carbohydrates or starches. We found that of these the diastases were the most important, and that by their action starch was converted into a soluble sugar—whether it existed in the form of a vegetable starch or an animal starch. We found that cellulose was likewise attacked by an enzyme which is particularly of vegetable origin—the cytase—and that, as in the case of other carbohydrates, the cellulose was converted into some kind of soluble sugar. The final product of the action of the diastatic enzymes and their allies is in each case a sugar, though the sugar formed may not always be the same. It will be convenient here to consider very briefly another class of enzymes which act, not on starch, but on the formed sugar, and which may be termed sugar-

splitting enzymes. Such enzymes, like the saccharifying enzymes, are to be met with both in animal and vegetable organisms. We will only refer to two typical examples—*invertase* and *maltase*. First as regards *invertase*. It was found that cane sugar can be split up by the action of acids into a mixture of two other sugars, glucose and fructose. Whilst cane sugar has the property of rotating a ray of polarised light to the right, the decomposition product turns the beam to the left. The process of the decomposition of cane sugar has therefore been termed *inversion*, the new product formed *invert sugar*, and the enzyme, which it has been proved can set up this change, *invertase*. The enzyme *invertase* was first discovered in the yeast cell. Cane sugar is not fermentable by yeast, but the invert sugar arising from it is. The yeast produces an invertase which can convert cane sugar into a fermentable form, *i.e.* capable of undergoing the alcoholic yeast fermentation. This invertase can be isolated from the yeast cell, and behaves then like an ordinary soluble ferment or enzyme. It is likewise present in a number of moulds, and appears to be formed also by bacteria. The higher plants similarly produce it, whilst it has been found to exist in the small intestine of animals and gives rise to reducing sugars from the cane sugar. This is important, as cane sugar *per se* is of no use for purposes of nutrition in the blood, but is converted into a nutritive form by the action of the intestinal invertase. This discovery was made by Claude Bernard—viz. that the animal organism is not able to assimilate cane sugar as such. He was led to think that the same might prove to be the case as regards vegetable protoplasm, and he accordingly

made experiments with the beet. The juicy tissues of the root of the beet contain a large amount of cane sugar. In the course of the growth of the stem and leaves of the beet the cane sugar in the root diminishes and becomes less as the growth proceeds. At the same time glucose appears in the growing portion of the stem, but no cane sugar. He succeeded in isolating from the beetroot an inverting enzyme, an invertase, similar to that found in the intestine of animals. Therefore, in the vegetable as well as in the animal organism cane sugar requires to be inverted into a reducing sugar before it can be utilised as food by the vegetable cells. Invertase has, therefore, important functions and is widely distributed amongst plants. The enzyme has great power; it has been found to be still active after inverting 100,000 times its own weight of cane sugar. The function of invertase in the animal and vegetable economy, therefore, is to convert cane sugar into other sugars which can be assimilated by protoplasm.

We now pass to the second example of a sugar-splitting enzyme, known as *maltase* or *glucase*. Maltose is a sugar closely related to cane sugar in composition, and, like it, is not as such utilisable by protoplasm, as is the case with *glucose*. It would appear that in the digestion of starch in the intestine, maltose is first formed, and from it glucose by the action of a maltase enzyme. Maltase is likewise present in *yeast cells* as well as in other vegetable organisms, *e.g.* in maize, and, like invertase, appears to play an important part in the nutritional processes of a number of plants and animals.

These instances of the sugar-splitting enzymes will suffice; their cardinal function appears to be the

conversion of certain sugars into other saccharine modifications, whereby such sugars are rendered available for purposes of nutrition in the plant and the animal.

The glucoside-splitting ferments form another interesting group. The glucosides are complex bodies occurring in plants, and into their constitution some form of sugar enters, generally glucose; hence the name applied to them. Certain enzymes produced by plants attack and split up these glucosides, a typical example being *emulsin*, and its action on the glucoside *amygdalin*. This enzyme is found, for example, in the almond and in the cherry laurel. By its action on the glucoside, sugar and prussic acid are formed, and in this way nutritive products such as sugar are obtained for the plant. A similar enzyme exists in the mustard seeds, which has been termed *myrosin*. It is an interesting fact that mustard oil does not exist in the seeds, but is first formed on their contact with water. The action of the water is to bring the enzyme myrosin into contact with a glucoside, myronate of potash, which it splits up, one of the products being the mustard oil. A number of such enzymes exist, although in many instances their action has still been imperfectly investigated.

We noted that as regards the animal organism, the three main sources of food were the proteids, the carbohydrates, and the fats. We have dealt with the digestive processes occurring by the action of soluble ferments or enzymes on the proteids and the carbohydrates. It remains, in order to complete our survey, to say a few words with reference to the action of enzymes on the third group, the fats. Such fat-splitting ferments are termed *lipases*. The fats consist of a combination of glycerin with fatty acids,

which form neutral compounds, and the fat-splitting enzymes decompose the fats into their constituents—glycerin and free fatty acids. In the digestion of fat the pancreatic secretion plays an important part; it splits up fats with the production of free fatty acids. The free fatty acids unite with the sodic carbonate in the intestine and form a soap, which favours the emulsification of the rest of the fat—*i.e.* the conversion of the fat into very fine globules—and thus its assimilation by the cells of the intestinal wall is promoted. Such lipases have been found to exist amongst a number of animals, and are likewise to be met with in the vegetable kingdom. The seeds of plants store up fats just as they do starches as reserve materials, which become dissolved and disappear in the process of germination. This action has been proved to be due, as, *e.g.*, in the seeds of the castor-oil plant, to the action of a lipase, which converts the fat or oil into fatty acid and glycerin. The fatty acid is used for nutrition, and the glycerin may form the antecedent of some kind of sugar.

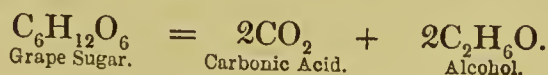
We see how versatile and varied are the means at the disposal of plant and animal cells for the manipulation of their foodstuffs, the main agents being soluble ferments or enzymes. We have now completed our general survey of the proteolytic enzymes, the diastatic enzymes, the sugar-splitting enzymes, and the fat-splitting enzymes.

In our next lecture we propose to consider the alcoholic fermentation and the interesting problems which its study has suggested, not only to chemists, but likewise to physiologists.

LECTURE V.

The Alcoholic Fermentation—The Germ Theory of Disease—Pure Culture of Yeasts—Employment of Pure Cultures in Brewing, &c.—The Alcoholic Ferment, Zymase.

In the present lecture I propose to consider the alcoholic fermentation in closer detail, as there is none which has been more thoroughly studied; whilst the problems which it has brought to the surface are of the greatest interest, not only to the chemist, but likewise to the physiologist. Indeed, the study of, and our knowledge of, fermentations generally are intimately bound up with that of the alcoholic fermentation. There is none which has been so carefully investigated either biologically or chemically. The essential features of the alcoholic fermentation consist in the conversion of sugary fluids into alcoholic beverages of various kinds, whether they be wines, beers, or spirits, the process being accompanied by a marked development of gas. In the course of the process sugar disappears, and spirit or alcohol appears. The chemical constitution of both the sugar and the alcohol being known, the process can be represented accurately by means of a chemical formula. The formula is as follows :—



This would be the ideal course of the alcoholic fermentation, viz. alcohol + carbonic acid, but in addition to alcohol other bodies are formed. Pasteur discovered that besides alcohol certain by-products appear; it is not a simple decomposition into alcohol and CO_2 . Of these secondary products he identified two, viz. the bodies glycerin and succinic acid, and besides these several other by-products occur in the course of the alcoholic fermentation. Thus, at the end of an alcoholic fermentation a certain amount of acetic acid is found to be present. We have likewise the fusel oils which occur in spirituous fermentations, but these need not detain us. To take typical examples: fruit sugar and grape sugar, as a result of a fermentation process, are converted into two principal products—a liquid and a gas, viz. alcohol and carbonic acid. Sugars such as cane sugar are not directly fermentable, but become so after a change in their chemical constitution which is known as inversion, and which is brought about by the action of enzymes, known as invertases. Such enzymes may be produced by the yeast cell itself, and to them we have already referred. This, then, in broad outline is the alcoholic fermentation, a process whereby sugar is converted into alcohol and carbonic acid. And this is the process as expressed in chemical terms; but when we come to inquire into the cause of the process we find that we cannot explain its origin on purely chemical lines, nor look upon it merely as an interaction of two chemical substances. Leeuwenhoek, as far back as 1680, with the aid of his simple magnifying glasses, discovered in fermenting liquids certain spherical bodies, but their exact nature and significance

remained for a long time unnoted by him and by others.

The possibility of the existence of a biological factor in the process was overlooked. The celebrated chemist Gay Lussac considered that oxygen was the essential factor in the initiation of the fermentation process; and this view was the one that prevailed for a very long time. At the beginning of the last century the discovery was made that the spherical granules observed originally by Leeuwenhoek were of an organised nature, and were in fact vegetable organisms or vegetable cells. Attention was therefore once more attracted to them. Schwann was one of the first to disprove Gay Lussac's oxygen theory of the alcoholic fermentation.

Schwann found that air, which, of course, contains oxygen, when heated before admission to the fermentable fluid, did *not* set up any fermentation of the same, although the heated air remained chemically unaltered. There was, therefore, something present in the ordinary atmospheric air which produced the decomposition of the fermentable liquid and which was not an ordinary chemical body. This factor, through its destructibility by heat, was probably of the nature of a living cell or germ—something vital. The essential factor in the process was *not*, therefore, the oxygen of the air, as Gay Lussac had supposed. But the proof of this vital, as opposed to a chemical, theory of the fermentation had yet to be supplied; and the vital theory till this proof was ultimately furnished was strongly attacked and ridiculed by many distinguished observers. One of the most distinguished chemists of his time—Liebig—elaborated a mechanical theory of the fermentation

process, viz. that substances which are in a state of active chemical change, are at the same time in a condition of active molecular movement, and that this movement is readily transmitted to other bodies with which they are in contact. Thus, the alcoholic fermentation would be due to a chemical decomposition of the ferment substance, or, as we now term it, the yeast cell, transmitting its instability to the sugar. Observations, however, increased in number, which showed the untenability of a purely mechanical or chemical theory of the origin of the fermentation process. And, finally, the investigations of Pasteur gave conclusive proof of the correctness of the vital theory of the alcoholic fermentation. There is a something present in the air which can be destroyed by *heat* which is of the nature of an organised solid body. As a further proof, it has been found that a simple and adequate filtration will remove from the air its capacity of inducing fermentation. Thus, if a sugar solution or any fermentable fluid be sterilised by heat and placed in a flask which is stoppered with cotton-wool, the fluid remains unaltered, though there is free admission of air, which, however, has been filtered by passage through the cotton-wool. On the cotton-wool a deposit is present which, examined microscopically, is found to consist of organised particles; these, if added to the fluid, produce the same changes as unfiltered air. The fermentation of the saccharine fluid is therefore an infection from the air by something suspended in it—viz., minute unicellular vegetable organisms known as the yeast cells. If a flask containing the sugary fluid be well boiled and stoppered while boiling, no change in the fluid ensues. If to it filtered air be admitted, also no change occurs;

while the unboiled fluid, or the boiled fluid to which unfiltered air is admitted, undergoes the change constituting fermentation, and in the fermented liquid organised germs are found. If the deposit on the filter be added to the boiled fluid, the fermentation changes follow. Therefore, fermentation is due to the particles of the deposit; it is an infection. This fermentation is accompanied by a multiplication of the germs in the fluid, and the multiplication of the germs is coincident with the fermentation. These germs are the yeast cells, and therefore the alcoholic fermentation is due to a multiplication of the yeast cells. Exclude them and it does not occur, admit them it does; it is a physiological act. To summarise these observations :—

1. Always where vegetating yeast cells are found, sugar decomposes according to the formula of the alcoholic fermentation—viz. into alcohol and carbonic acid.

2. Wherever alcoholic fermentation occurs, the living yeast cells are to be found.

3. Both phenomena are, therefore, intimately and essentially connected.

4. And the conclusion must be formulated that the alcoholic fermentation is a physiological act on the part of the organism in question.

What is the rationale of the process? The yeast takes sugar as food, and in the course of this process separates alcohol and carbonic acid. There is no spontaneous generation of the yeast, since a sterilised fluid remains unchanged, or changes only on addition of a yeast cell. And this yeast cell is derived and produced from a previously existing yeast cell.

This is the outcome of Pasteur's great work on the vital theory of fermentation, and I may be allowed to refer to its wide significance. From it the germ theory of disease has developed. It was found that there are living agents in infective diseases, which can be detected by the microscope and be cultivated outside the body. These living agents, if reintroduced into a healthy animal, reproduce the same disease. Thus, in the disease known as anthrax there is a multiplication of anthrax bacilli in the tissues and blood, and just as the alcoholic fermentation is an infective process, so an infective disease is likewise an infective process. Antiseptic and aseptic surgery, which we owe to the genius of Lister, consist in an exclusion of the infective agents, so that the surgeon's wounds heal rapidly and normally.

To return to the alcoholic fermentation, it is due to vital agents—the yeast cells. These yeast cells are vegetable cells, of which the typical example is brewer's yeast, known scientifically as the *Saccharomyces cerevisiæ*, and it multiplies by gemmation or budding (fig. 10).

Of yeasts there are various species, of which some are good and useful to man, some are bad, and they respectively produce a pure or an impure alcoholic fermentation. In the old rule-of-thumb methods of brewing a mixture of yeasts was frequently present, and the resulting alcoholic fermentation was frequently bad, impure, or imperfect, and a bad beer or a bad spirit was the result. In order to get over this difficulty, which often entailed great loss to the producer, the principle of pure culture was adopted, which we primarily owe to Koch, Brefeld, and others.

By this method suitable nutrient soils or media are prepared, such as sugar solutions, infusions of malt (wort), and the like, and the media are rendered sterile by boiling; all living organisms that may be present in them are killed by the boiling. By then transferring single cells to the sterile culture media thus prepared a growth of yeasts or other organisms *of a single species* ensues—a ‘pure culture’ results. This implantation of single cells in the sterile

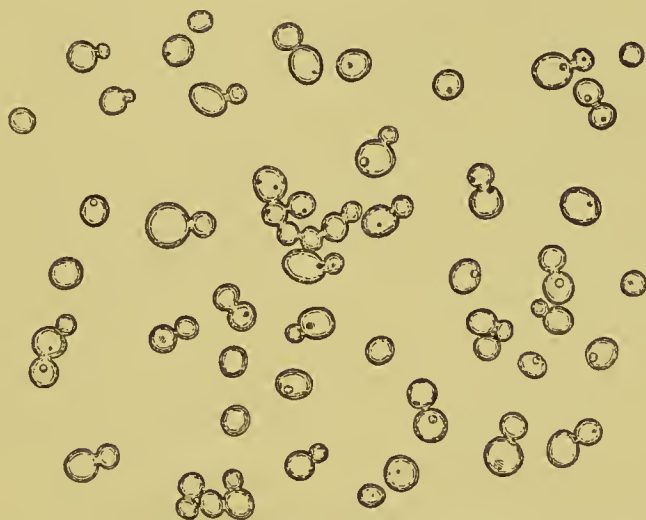


FIG. 10.—Yeast cells showing budding.

media may be accomplished by highly diluting the original yeast and transferring traces of this dilution to the prepared media or in other ways. In the case of the yeasts the preparation of pure cultures was first accomplished by Hansen, of the Carlsberg Brewery, Copenhagen, and his work has revolutionised the brewing industry.

When Hansen commenced his work the brewing industry was conducted empirically by rule-of-thumb

methods, and mixtures of yeast were frequently used, and the beer produced was sometimes bad owing to the wrong kind of yeast growing in the wort. Hansen established a botany of the various kinds of yeasts, and isolated the good from the bad species, prepared pure cultures of them, and grew them in pure cultivation.

The results of Hansen's researches may be summarised as follows :—

1. There are several botanically distinct species of alcoholic yeasts. To this group the term or generic name *Saccharomyces* is applied—*i.e.* they can reproduce in its main features the alcoholic fermentation.

2. Alcoholic fermentation is due to the vegetative growth of the yeast cells in the fermentative liquid.

3. The value of these various yeasts as producers of alcoholic fermentation varies, some being better than others.

4. There are specific forms of yeast cells—that is to say, there are special forms for the beer fermentation, special forms for the wine fermentation, &c.

5. In technical processes the aim is to select the good forms of yeasts, and to separate them from the bad forms, or 'wild yeasts' as they have been called.

6. For this it is necessary to use pure culture methods in order to obtain the right strains for seeding purposes. This Hansen succeeded in doing.

We have now come so far as to be able to say that

1. The alcoholic fermentation is the formation of alcohol and carbonic acid from sugars.

2. Together with this there are various by-products, *e.g.* glycerin and succinic acid.

3. The alcoholic fermentation cannot be explained on a purely mechanical theory, *e.g.* Liebig's theory.

4. The oxygen theory is equally untenable, through Schwann's experiments with heated air.

5. Pasteur demonstrated the truth of the vital theory of alcoholic fermentation.

6. Fermentation is due to vegetable cells, the yeast cells—

a. Wherever alcoholic fermentation occurs yeast cells are found.

b. Wherever yeast cells gain access to a saccharine fluid we have an alcoholic fermentation.

7. The vital theory is therefore conclusively proved.

8. Hansen's researches defined a botanical group of yeasts, the *Saccharomyces*, some of which are good and useful; some are bad, and cause improper fermentation.

9. Hansen rendered the cultivation and utilisation of useful breeds possible. This he did by means of pure cultures.

We noted that the alcoholic fermentation was due to various yeasts, *e.g.* the wine and the beer fermentations. But in some cases the resultant process is due to an admixture of organisms, to a symbiotic fermentation, as it is termed. Such, for example, are—

1. *Koumiss* from milk, the agents being (*a*) alcoholic yeasts, (*b*) lactic acid producing bacteria.

2. *Kephir* from milk, the agents being yeasts and bacteria.

3. The *Ginger-beer plant* is a mixture of yeast and a bacterium, the result being alcohol and acids.

4. *Koji*, the agents being a mould and yeasts.

5. *Chinese yeast*, the agents being a mould and yeasts.

Other cells can produce alcohol from sugar besides yeasts, *e.g.* a number of bacteria. Thus, some of the colon group of organisms, organisms isolated from the small intestine, can effect this.

We have now traced the chemistry and biology of the alcoholic fermentation.

The yeast cell is the prime agent in the fermentation. The question still remains, By what method does the yeast cell effect the decomposition of the *sugar*? Our previous lectures have shown that—

1. *Proteolysis* is due to soluble ferments—pepsins, &c.

2. *Curdling* or *clotting* of proteids is due to soluble ferments—rennet, &c.

3. *Saccharification* of starch, &c., is due to soluble ferments—diastases, &c.

4. *Splitting up of fats* is due to soluble ferments—lipases.

5. Even *inversion* of sugar into fermentable forms is due to an enzyme—invertase.

All these processes are therefore due to soluble ferments or enzymes, unorganised ferments.

But it had hitherto not been possible to separate an alcohol enzyme from yeast cells, and therefore the alcoholic fermentation by yeast was regarded as an act of its living protoplasm bound up with the life of the cell. Hence the distinction which has been made between (1) the unorganised ferments and (2) the organised ferments, the latter being really all those fermentations in which an enzyme has not been demonstrated.

We have seen how invertase at first appears to be an organised ferment. But if the yeast cell be injured, it passes out and acts as a soluble ferment; invertase, therefore, is of the nature of an enzyme. If, then, yeast produces a trypsin and invertase, why should there not be an alcoholic ferment? To this question much study has been devoted.

As already stated, the alcoholic fermentation was looked upon as indissolubly bound up with the life of the yeast cell; that the process was a purely vital one—a metabolic process on the part of the cell itself. And an experimental proof of the contrary, viz. that it might be likewise due to the action of an enzyme, was lacking. This proof was claimed to have been furnished by Buchner in a remarkable series of experiments he conducted. In 1897 Buchner published a communication in which he described a method by means of which he claimed to have isolated for the first time the active alcoholic ferment from the yeast cells, and to have demonstrated its action on fermentable sugars. Since then Buchner has from time to time given an account of his further investigations in this direction, and these investigations are still in progress. These further investigations, Buchner considers, are confirmatory of the conclusion drawn by him from his original experiments—viz., that the activity of the yeast cell as an alcoholic ferment depends upon the action of a soluble enzyme of an albuminoid character elaborated by the living cell. To this soluble ferment Buchner applies the name ‘zymase.’

This would range alcoholic with the other fermentations we have already studied, as due to a soluble

ferment or enzyme, produced by the living protoplasm. How did Buchner reach this conclusion, and what were the methods he applied?

We saw in the case of invertase that a weakening of the cell by chemical agents, &c., detached the enzyme from the living cell.

Buchner used still more drastic methods.

If the alcoholic ferment were an enzyme, it evidently is firmly anchored to the cell protoplasm of the yeast. Therefore the cell juice of the yeast cell, or cell plasma, must be obtained. To do this he completely broke up the yeast cell—by no means an easy matter with so small an object.

In his method purified pressed yeast was mixed with an equal weight of fine quartz sand and one-quarter of its weight of kieselguhr (infusorial earth). The mixture was ground up in a mortar, producing a moist plastic mass, in which the microscope showed that the cells were disintegrated or ruptured. This plastic mass was then diluted with distilled water, wrapped in cloth, and submitted to pressure in a hydraulic press, and this process was repeated several times. This resulted in a cake and a squeezed-out liquid. The liquid was filtered, and formed an opalescent, yellowish fluid, having a yeasty odour. If to this fluid sugar were added, carbonic acid was formed in thirty minutes, and a certain quantity of alcohol was also produced, chloroform or toluol being added to prevent the growth of any yeast cells which had escaped disintegration. The inference from this result is therefore that the alcohol is due to an enzyme secreted by the yeast cells.

Is this zymase really the alcoholic ferment or enzyme? On the whole the evidence is in favour of it really being so, although it has been suggested that fragments of living protoplasm may be the active agents. In any case these researches lead us nearer to living protoplasm — this enzyme, the zymase, is more intimately associated with living protoplasm than is the case with other enzymes — and open up a whole field of intracellular physiology.

LECTURE VI.

Oxidising Ferments, Oxidases—Diabetes—Acetic Acid Fermentation—Lactic Acid Fermentation—Nitrification—Putrefaction—Technical Importance of Fermentations—Summary.

Enough has now been said to illustrate the great interest attaching to the study of the alcoholic fermentation. The yeast is a most remarkable and interesting cell, and its study brings us into contact with physiological problems of the widest bearing as regards both animal and vegetable life.

There are certain fermentative processes which still remain to be touched upon. Recently research has been bestowed upon the enzymes known as *oxidases*. These promote the direct oxidation of various substances and are widely distributed, occurring both in plants and in animals. They act as carriers of oxygen, and therefore set up oxidative processes. They probably have important functions in metabolic processes.

First, as regards the oxidases met with in animal tissues. They are substances which reduce hydrogen peroxide (H_2O_2) to the condition of water and set oxygen free, and in this way oxidative processes can be brought about by the nascent oxygen. For instance, organ extracts in the presence of oxidisable substances produce an oxidation of the same, as may be shown by the bluing of tincture of

guaiacum, and such oxidative processes have been ascribed to the action of enzymes. The following classification of oxidising agents has been given :—

1. *Ozone* can part with and give up oxygen or pass it on, *e.g. per se* can blue tincture of guaiacum.

2. *The Ozone Carriers, e.g., chinon*, which act in virtue of the ozone they contain.

3. *Genuine Oxidases*.—Differ from group 2, or ozone carriers, inasmuch as their oxidising properties are not dependent on the given amount of the oxidising agent they may contain. They are genuine ferments, and promote the transference of oxygen so long as their ferment action is not destroyed by heat, &c., or other agents inimical to ferments.

4. *Substances* which only exercise oxidative function in presence of hydrogen peroxide. They use hydrogen peroxide as a source of oxygen and are termed ‘indirect oxidases.’ Their action is likewise destroyed by heat.

The following animal oxidases may be alluded to :—

1. *Salicylase*, which oxidises salicylaldehyde to salicylic acid.

2. An oxidase causing the conversion of xanthin into uric acid.

3. *Oxidases* which blue tincture of guaiacum.

In blood which has been shed the sugar disappears. This is supposed to be due to an enzyme, and suggests the presence of an oxidase. In other words, a destruction of sugar takes place, probably due to the action of an oxidative enzyme or oxidase. It is also supposed that the pancreas secretes such a sugar-destroying or glycolytic ferment. Such a substance might have a bearing on the pathology

of diabetes, in which disease there is an excessive excretion of sugar. It is suggested that in health the pancreatic secretion destroys any excess of sugar; but that in diabetes the pancreas is diseased and the sugar-destroying ferment is lacking, and hence any excess of sugar is not destroyed, but is excreted, and the condition of diabetes obtains.

In plants oxidases are likewise to be met with. For instance, there may be changes in the colour of plant pigments. One of the earliest to be studied was laccase, which is concerned in the production of lacquer varnish from the crude sap of the lac tree, grown in South-East Asia. The crude sap of the tree is a creamy liquid which, on exposure to the air, becomes a brown and finally a black colour. The sap contains a substance, uristic acid, and also an enzyme, laccase, which converts the acid into the varnish.

There are in certain fungi pigments which on exposure to air become red and finally black, and in another case a fungus, when broken and exposed to the air, becomes blue. Both these changes are due to oxidases. Further, wine when kept sometimes undergoes deterioration, manifested in a discoloration; this may also be due to an oxidase. Similarly the discolorations which occur on the surface of fruits—*e.g.* a cut apple, which becomes reddish brown—may be due to oxidation of the tannin by an oxidase, and many other instances might be given. The exact enzyme character of a number of these oxidases has not, however, yet been accurately proved.

There are still certain other oxidative fermentations to be considered, which seem to be intimately bound up with the action of the living protoplasm.

A typical example is the acetic fermentation. When dilute alcohol is exposed to air it gradually becomes sour and the cause of this was long a matter of discussion. Liebig applied his mechanical theory in explanation of it. Then Pasteur and his pupils advanced the vital theory, which proved to be the right one, for the change is due to the action of micro-organisms. In the process a pellicle forms on the surface of the liquid, and the liquid may also become viscid.

The process consists in a conversion of the alcohol into acetic acid by oxidation. It is due to vegetable cells, which gain entrance from the air. The most common of these is the *Mycoderma aceti*, an organism which belongs to the group of the bacteria, and there are several other organisms possessing similar properties. Thus, the so-called vinegar plant is a bacterium, and it produces likewise acetic acid from alcohol. The well-known souring of milk is a lactic-acid fermentation, due to the conversion of the milk sugar into lactic acid by the action of bacteria.

The decay of organic matter in soil in the final stages largely results in the formation of ammonia or its compounds from the nitrogenous matters present. It was formerly supposed to be purely chemical in nature. At first the complex organic matter is broken down with the formation of compounds of ammonia, and these again are converted first into nitrites and finally into nitrates. The latter changes are known as nitrification, and are biological changes brought about by two distinct groups of organisms which set up the process—viz. nitrous and nitric organisms, both of which are present in the soil.

Nitrification is, of course, of the greatest importance in agriculture, and without micro-organisms would not occur.

Putrefaction, the resolution of organic matter into simpler substances, is likewise due to bacterial action, and is also most important in Nature. Without putrefaction the earth would be encumbered with the remains of dead animals and plants.

In all the above instances the direct intervention of the living cell appears to be necessary in order to bring about the respective changes. In the changes which occur in tanning, in tobacco fermentation, and many other instances, micro-organisms play a great part, and are therefore of the utmost technical importance and interest.

We have now concluded our general survey of the main types of fermentation processes and the agents whereby these are brought about—the living cell or some soluble product of the same. We have noted the distinction drawn as regards fermentative agents between the organised and the unorganised ferments, the organised ferments being living cell protoplasm, the unorganised ferments being the soluble products of cell protoplasm—the enzymes. The conversion of food into a soluble or assimilable form is a paramount function of enzymes.

In this connection we traced the action of enzymes on the main groups of foods—proteids, carbohydrates, and fats. As regards the *proteids*, we have proteolytic enzymes, such as *pepsin*, present in the gastric juice, acting in an acid medium and resulting in the formation of

peptones ; and *trypsin*, present in the intestine, acting in an alkaline medium and resulting in the formation of leucin, tyrosin, and peptones.

These proteolytic ferments are widely distributed in the animal and vegetable kingdoms, in the latter occurring in insectivorous plants and in the pineapple.

Then there are clotting enzymes, which also act on proteids. Such are *rennet*, which occurs in the calf's stomach, and curdles the casein of milk, and is an essential in cheese-making ; the *blood-clotting* ferment—fibrin ferment—which acts on the fibrinogen of the blood and forms a clot of fibrin ; the *muscle ferment*, which clots the myosin of muscle and causes *post-mortem* rigidity of muscle ; and there are likewise clotting ferments to be found in the vegetable kingdom.

Next there are the saccharifying enzymes, such as the *diastases*, which convert starch into sugar.

Starch is a food reserve found in the plant, and a similar food reserve is found in the animal in the form of glycogen or animal starch. The insoluble starch is changed into a soluble, diffusible, and transportable sugar, which can be used in the economy of the plant or animal organism. The saccharifying enzymes play the same rôle as regards carbohydrates as that played by proteolytic enzymes as regards proteids. They are engaged in furthering the nutritive processes of cells, and are likewise important in technical processes in bringing about a change of starchy matter into sugar which can be fermented by the yeast cells.

We likewise touched on cellulose-dissolving enzymes, which render cellulose available for building up the frame-

work of the plant; these are the *cytases*. All the above are soluble ferments or enzymes. We then discussed the alcoholic fermentation, which must be regarded as a phase in the nutritive processes of the yeast cell. In this connection the 'enzyme theory' of Buchner and the enzyme or ferment *zymase* were considered. Finally we touched upon the *oxidases*, which act as transmitters of oxygen and occur notably in plant cells, and alluded to those fermentation processes which appear to be intimately bound up with the direct intervention of the living cell, such as the acetic fermentation, nitrification, and putrefaction, in all of which bacterial agents are at work.

We also touched on the analogy existing between fermentation processes and the intoxications of animal organs and tissues due to toxins elaborated by bacteria, notably in diphtheria and tetanus. The infective agents act not directly, but by means of soluble products or toxins which they elaborate at the seat of infection. These toxic bodies share many of the properties of the enzymes, and appear to be closely allied to them. They act independently of the cells that produced them, and are toxic in extremely small doses. The study of the enzymes or soluble ferments has therefore not only a physiological, but likewise a pathological, bearing.

The main problems of animal and plant life are therefore intimately associated with a knowledge of the nature and mode of action of the ferments or enzymes we have been considering. Every ferment, as we have seen, acts specifically on certain groups of chemical substances—

e.g., the proteolytic on proteids and the saccharifying on carbohydrates.

The reason for this is difficult to explain. It probably depends on some similarity in molecular structure between the enzyme and the body it attacks. The key must fit the lock.

Are the enzymes simply centres of certain forms of energy, or are they genuine chemical bodies? Most observers consider them to be definite chemical bodies, but their exact nature is still unknown, since no chemical analyses of them have yet been possible; they have no distinctive chemical tests, and we know them only by their mode of action.

It is true they can be extracted and also precipitated, but they are very sensitive bodies—sensitive to the action of physical agents, such as heat and light; to chemical agents such as acids and bases, which, if strong, may destroy their activity, but if weak may aid their action. Proto-plasmic poisons do not, as a rule, affect their activity; thus they act in the presence of chloroform, thymol, carbolic acid, &c. The activity of the enzymes is remarkable. For example, one part of rennet curdles 400,000 parts of casein; one part of invertase changes 100,000 parts of cane sugar; and the fatal dose of tetanus toxin is 0.00023 gram.!

The enzymes, though in the higher plants and animals formed in glands, are but special products of the *cells* of the glands, and are formed equally well by unicellular plant and animal organisms. Their importance for life processes has been demonstrated; they are intimately bound up with assimilation and nutrition and with the

building up of the cell protoplasm. Whilst they must not be confounded with the specific vital forces of the cell, they essentially minister to its life and support all vital functions.

This closes our survey of the enzymes or ferments, which, I think, has shown them to form one of the most important chapters on Cellular Physiology.

RECENT METHODS AND RESULTS IN
BIOLOGICAL INQUIRY

A COURSE OF THREE LECTURES DELIVERED AT
THE ROYAL INSTITUTION

APRIL 1902

LECTURE I.

The Place of Hypothesis in Scientific Inquiry—The Cell and the Cellular Doctrine—Methods of Examining Cells and Tissues—The Microscope and Photomicrography—The Bacteria—The Protozoa—Physical Factors modifying Vital Processes.

It will be obvious that to attempt a survey of a territory as broad and varied as life itself is an impossibility, and that it is equally impossible to do justice to the harvest of results that has been gathered in recent years in the field of biology. We can at most hope to direct attention to some of the recent methods and results in this field as they appeal to an individual worker with necessarily specialised aims, but at the same time with the desire to ascertain in how far his own and other lines of research are in harmony with the general trend of biological inquiry.

Whatever our occupation, we act and work, consciously or unconsciously, under the influence of the dominating ideas peculiar to our age. Even Science must live by faith in its own hypotheses. The doctrines of the correlation of the physical forces, of the conservation of energy, and of evolution have influenced the most specialised branches of investigation. The theoretical speculations of Maxwell inspired the experimental methods of Herz, leading to the birth of

wireless telegraphy and its practical realisation by Marconi and others. The conservation of energy, at first a purely physical doctrine, has been extended in application to living as well as to lifeless matter, whilst the principle of evolution animates every phase of vital inquiry. There is happily an increasing appreciation of the solidarity that exists between the various branches of knowledge. The exact sciences of physics and chemistry have joined hands, and the methods of physical chemistry are already influencing the study of vital problems. The chemist and the physicist, on the other hand, are finding in biology many results worthy of their closest attention. To work as a specialist is necessary; to think as a specialist may be dangerous. The most fruitful results will be obtained in the future as in the past by those who are fortunate enough to trace the unity that exists under the apparent diversity of phenomena. The high type of mind that is able to co-ordinate and to synthesise facts is a priceless boon to the rank and file of workers.

It cannot escape our attention that with regard to the subject-matter of this course of lectures, the cell at the present moment occupies the forefront of investigation. The essential functions of life are exhibited by the free-living unicellular organisms of the animal and plant worlds. The higher forms of life are aggregates or colonies of cells, and in the highest example, man, the organs and tissues are framed of cellular elements which structurally resemble the simplest organisms to be met with in Nature. The cell is the unit of life and the physical basis of all vital phenomena. The conception of the cell as the physical basis and structural unit of

life has been arrived at by a close microscopical study of the tissues of plants and animals. The interpretation of the term has varied from time to time, and the definition now adopted has rendered it inappropriate and somewhat misleading. The expression, however, is so convenient that it has been generally retained. The term implies a walled-in cavity, as it was originally supposed that a confining wall or membrane was a constant and essential constituent of the cell. In the plant a cell wall is an obvious and prominent feature; but in the animal kingdom a large number of unicellular organisms, as well as the cells composing the tissues of the human body, are destitute of an enclosing membrane. An outer membrane is not therefore an essential factor in cell life. The primitive type is the membraneless cell, consisting of a semi-fluid protoplasm with a contained body—the nucleus. The structural unit of life is a minute speck of protoplasm with a nucleus. The properties which reside in the protoplasm and the nucleus are necessary to the entire fulfilment of the life processes of the cell. Each element influences and is incomplete without the other, and the death of the cell ensues if their connection is severed. The nucleus is a determining factor in many of the cell's activities, and notably during the reproductive phase of its life. This reproduction is brought about by the division of a cellular unit into two daughter cells. The common factor in all forms of cell multiplication is a transference of nuclear as well as of protoplasmic substance to the daughter cells. The nucleus plays an important part in the initiation of the process, during which the cell preserves its characteristic structural elements. The nucleus

is likewise of importance in the maintenance of function, and is essential, for example, to the digestive processes of many cells, whilst a non-nucleated cellular fragment will die. There must, therefore, be a constant chemical interchange occurring between the protoplasm and nucleus of the cell, and both elements are essential to ensure its self-preservation (see also p. 69). Every cell has its origin in a pre-existing cell—*omnis cellula e cellulâ*.

The definition just given holds good for the cellular elements of animal and vegetable tissues. There is a structural identity in the physical basis of plant and animal life, and in each instance the cardinal vital processes are of a similar character. The structural and functional unit of life has thus been determined in so far as our present methods render this possible. The essential phases of life can be studied in the humblest unicellular organism and valuable information obtained regarding the properties of cells generally, more particularly as regards the action and reaction occurring between an organism and its environment. The essential vital phenomena being identical in the plant and animal cell, and possessing a common physical basis, it must be obvious that the main physiological problems are ultimately cellular problems.

Refined microscopical methods have revealed in the case of the complex and higher organisms a cellular structure of all their parts—cells are the bricks of the living tenement. The study of purely anatomical relationships is being largely replaced by the study of the intimate structure of the tissues and their cellular framework. Embryology is essentially a cellular study; the

reproductive activities are based on and resolve themselves once more into cellular elements.

The study of the normal processes of life is at the present time based on the cellular doctrine, and the pathological processes occurring in the body are in large measure regarded as perverted or degraded cellular activities. This broad theoretical conception dominates every branch of biological investigation.

The advances that have been made in methods and in laboratory technique have contributed notably to this result.

Physical methods of investigation in physiology have reached a high degree of refinement and exactness with regard, for example, to the effect of stimuli on muscle and nerve. The possibilities of such methods have been tested in almost every likely direction. The laws of dynamics and hydrostatics apply to the movements of the body and its fluids, and those of light and sound to the organs of sight and hearing. The human body regarded in the light of a machine is now pretty well understood. There are, however, limits to the knowledge that can be gained by research in this direction. The activities of life, whether evinced in muscular and nervous energy, or in digestive, assimilative and secretive processes, or in the perceptive power of the special organs of sense, originate in the cells of which the specific tissues and organs are composed.

This broad physiological conception has led to an increased application of experimental methods directly to the cell. The plant cell is in this respect more readily accessible to direct observation. To anyone desirous of

acquiring a knowledge of biological principles, there can be no better method recommended than the study of plant physiology. The essential identity of plant and animal protoplasm as regards their fundamental properties has been established, and physiological botany is therefore of the greatest value in the study of general biological questions. It will be sufficient to mention the observations made upon the behaviour of plant cells under the influence of various chemical stimuli, and the explanation that was thus afforded of the migration of the leucocytes of the human body in given directions (positive and negative chemotaxis, see p. 117). The protozoa likewise furnish convenient objects for the study of the action of stimuli generally upon animal cells.

The cell is a microscopical object and beyond the range of ordinary vision. The microscope is thus indispensable in the study of the cellular unit, whether existing free in Nature or fixed in the tissues of plants and animals. Inasmuch as there is little optical differentiation between the tissues and their cellular constituents in a complex organism, the use of the microscope is necessarily supplemented by the application to the objects under observation of various chemical reagents. Where a direct observation of the living cells is impossible, staining methods are required to bring into view structural elements and to differentiate the cell plasma and its contents. The remarkable developments of colour chemistry have greatly aided and forwarded microscopical work in this respect. The aniline dyes are now as important adjuncts in biological research as they are in the textile industries, and are constantly finding new applications in

the laboratory. Microscopical technique has undergone a corresponding refinement, more particularly in the direction of differential staining methods. The affinity of the acid and basic aniline dyes for different constituents of the cell—protoplasm and nucleus—yields at once valuable differentiations under the microscope, and aids in the detection of normal and abnormal conditions of the cell substance. By the admixture of various dyes double and triple staining effects can be produced as regards the nucleus, protoplasm, and contained granules. The study of the white cells of the human body has by such methods been placed on a new basis, a more accurate classification of the different forms of these cells has been made, and a fuller knowledge of their behaviour in health and disease has been obtained. The main advances in hæmatology (the study of the blood) are due to the application of methods in which the staining properties of the aniline dyes are made use of.

The basic aniline dyes are indispensable in the study of micro-organisms, as they stain pre-eminently these minute objects. The detection of bacteria within the animal body is rendered a comparatively easy matter, as well as their location in the tissues in which they occur as parasites. It is by such methods that the finer organisation of the cell is being studied, and there can be little doubt that in course of time protoplasm will be found to possess a definite structure of its own. The theories of the constitution of the nervous system have been revolutionised by the improvements in technique—the theory of neurons or isolated nerve elements having replaced the old theory of the intimate union of the nerve cells.

The application of micro-chemical reactions in recent years has proved of great value in determining the nature of the cellular substance and its contents. It will suffice to mention, by way of illustration, the detection by chemical reagents of such substances as starch, glycogen, cellulose, fats, and inorganic crystals. A number of these reactions can be readily watched with the aid of the microscope, and their use is indispensable in many branches of inquiry.

The low powers of the microscope reveal the relations of parts; the coarser features of the tissues and their cellular organisation. The highest powers of the microscope are required for the observation of the finer details of the tissues, as well as for the detection and study of the unicellular organisms, where the standard of measurement is the $\frac{1}{1000}$ part of a millimetre ($\frac{1}{25000}$ of an inch). The modern microscope has been gradually developed to meet these demands and has become an instrument of the greatest precision. A microscope furnished with fluorite systems, apochromatic lenses, oil immersion objectives, and an Abbé or other condenser opens out hitherto inaccessible fields of observation to the student, as a high magnification is attained without a resultant loss of definition. Photomicrography has made corresponding advances, and the permanent objective records of individual observations that can be obtained are of the greatest value for the purpose of illustration or reference.

It is by methods of the above nature that the cell has been recognised to possess a definite structural organisation upon which its functions rest.

There has likewise been brought to view a world teeming with microscopic forms of life in which the individual is represented by a simple cell. The bacteria belong to this category of unicellular organisms and are of so minute an order that a test-tube may contain numbers far exceeding the human population of the earth. Our knowledge of the valuable functions performed by these organisms in the economy of Nature is of comparatively recent date, and their study is at the present moment one of the most important chapters of biological inquiry. It is a remarkable achievement to have brought these infinitesimal objects within the range of observation and experiment, and to have devised methods for their isolation and cultivation in the laboratory. The activities of the bacteria are in the main of a beneficial and useful character, with the exception of a small minority which are capable of producing disease processes by their invasion of the animal body. Their chief function, however, is to clear out the dustbin of Nature by resolving the *débris* of animal and of vegetable life into simple and innocuous compounds. Many fermentations of technical importance are due to their action; milk and cheese have a bacterial flora of their own, whilst the nitrifying processes which contribute to the fertility of the soil are of bacterial origin. The great storehouse of bacteria is the soil, there they constantly minister to the life of every plant, and the continuance of human life ultimately depends upon the silent activities of these unicellular organisms in Nature. The bacteria are free-living cells, each in itself an independent unit. A cell must be free if it is to exercise certain important functions. The sanative value

of the leucocytes (white blood corpuscles), and the defensive properties of the phagocytes,¹ depend in large measure upon the mobility possessed by these cells. The deadly nature of the cancer cell lies in its power of detachment from the tissues and its elevation, if one may so express it, to the plane of a unicellular organism with migratory propensities. The fixed cells of the body, on the other hand, have altruistic duties imposed upon them—they serve not merely their own, but the higher and collective needs of the organism. The functions of the free cell are more or less in a condition of physiological balance; those of the fixed cell are usually highly specialised in a given direction, as, for example, in the organs of sense, digestion, secretion, &c.

The most important factors in our environment or body as regards our general well-being are the unicellular free lances—in the one instance the bacteria and protozoa, and in the other the white blood cells. The bacteria are vegetable cells. The protozoa furnish the most familiar examples of unicellular animal organisms. They consist of protoplasm with or without a membrane, with a contained body or nucleus, and are the morphological equivalents of the free wandering cells of the human body. The protozoon may be regarded as the primitive type of the animal cell, and its study aids in the interpretation of the more specialised cellular functions as they occur in the higher animals. The proteus animalcule or *Amœba* is a complete individual, and discharges all the essential functions of life—digestion, assimilation, secretion, reproduction, response to external stimuli, and, in addition,

¹ Wandering cells of the body which ingest bacteria.

possesses the power of free movement. Such organisms are widely distributed in Nature, and one of the results of modern investigation has been the differentiation of the protozoa from the bacteria and their classification as unicellular animal and vegetable forms of life.

A feature of wide biological interest is the process of intracellular digestion that occurs in the protozoa. The food is taken up directly and digested within the cell, and in some instances the process appears to be accompanied by the formation of an acid, as a reddening of absorbed litmus granules has been noted. Is this process analogous to that occurring in an acid fluid in the digestive tract of the higher animals? It may be that within the *Amœba* are contained the first evolutionary rudiments of human digestion. The protoplasm of the *Amœba* is alkaline, and the acid appears to be confined to the fluid in the gastric vacuole. The gastric vacuole may be described as a temporary stomach improvised by the *Amœba* as occasion requires. The protozoon ingesting and digesting the food within its cell substance

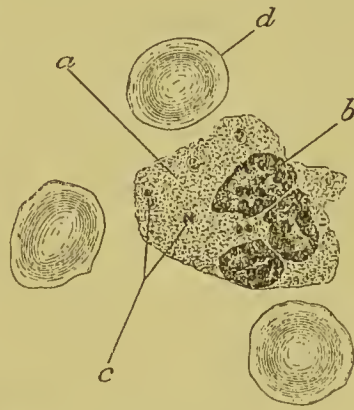


FIG. 11.—Phagocytosis. *a*, a polynuclear leucocyte with its nucleus, *b*, which has ingested several micrococci, *c*; *d*, red blood corpuscles.

is the prototype of the white blood cells of the human body, particularly of those which perform scavenging duties, and termed phagocytes (fig. 11). The process of phagocytosis appears to be a phase of the intracellular digestion that occurs in a large number of

unicellular organisms outside, as well as inside, the animal body. The study of the protozoa furnishes in this respect an important clue to the processes carried out by the wandering cells of the human body, to which so important a rôle has been ascribed in the protection of the tissues against the invasions of micro-parasites.

There are many questions of general physiological interest for which the unicellular organisms furnish convenient test objects. The effects of chemical and physical stimuli upon protoplasm can in this way be directly studied. A case in point is the phenomenon of chemotaxis or the motor responses of cells in the presence of certain chemical substances. The cells of every plant and animal are constantly under the influence of chemical stimuli, and the reactions that occur are of a vital character (see p. 117).

Certain phases in plant fertilisation are undoubtedly due to chemotactic influences, as well as the inflammatory exudations that occur in the human body. It is, however, only by means of experiments upon the simplest organisms that we are able to visualise the process, and to gain a clear conception of the agencies at work, and the positive or negative effects they are capable of producing.

Heat, light, and moisture are physical factors without which the active processes of life become dormant or cease, and the unicellular organisms once more present the simplest conditions for experimental inquiry. Light may be taken as a favourable example of the experimental results and the deductions it is possible to draw from them. Light of a certain intensity will attract and of a

greater intensity will repel unicellular organisms—in other words, the influence exerted by light may be of a favourable or unfavourable character. The rays with the most active properties are those of short wave length. These rays produce a cessation of normal protoplasmic movements, as, for example, in the *Amœba*. The red and the violet rays differ in their physiological effects, the actinic being the most unfavourable to living organisms. Sunlight possesses active bactericidal properties, due similarly to actinic radiations. The tubercle bacillus on exposure to direct sunlight is killed in a few hours. If it is desired to utilise the bactericidal effects of light, the radiations towards the ultra-violet end of the spectrum must be employed. These radiations likewise produce distinct effects on animal tissues. In man a solar erythema, or red rash, occurs, limited almost entirely to the non-pigmented areas of the skin, or a pigmentation may result which affords a certain amount of protection to the skin, as in the case of the negro. The acute effect consists in a superficial irritation, the chronic effect in a pigmentation, of the skin. An ordinary lamp has less, and an electric arc proportionately more, of these physiologically active rays than the sun.

The course of certain diseases is unfavourably influenced by light. The chemical rays act injuriously on the diseased skin of small-pox patients, but if their action be excluded, the tendency to local suppuration is lessened and almost invisible cicatrices result. The method of treatment accordingly adopted by Finsen consisted in the exclusion of the action of the chemical rays on the skin by keeping the small-pox patient in a room to which only

red light was admitted.¹ These rays, on the other hand, in moderation produce a valuable and healthy stimulation of the nervous system. The radiations towards the ultra-violet end of the spectrum have, therefore, distinct physiological properties, which vary according to the intensity of their action. The bactericidal action is slow, but it may be increased by the exclusion of the heat rays and by the use of various lenses. The result is a blue or violet light with active bactericidal properties, the best source being the sun or the voltaic arc. The rays when concentrated by means of a plano-convex lens kill micro-organisms much more quickly than when employed directly, and their therapeutic value is proportionately increased. Lupus is a superficial skin disease, due to infection with the tubercle bacillus. An exposure to light is, as already stated, fatal to the tubercle bacillus, and in lupus the best opportunity is presented for testing the effects of photo-therapy. It has been found that the violet rays applied daily for several weeks to a lupus patch produce a remarkably beneficial effect. Under their influence the edges of the lupus patch flatten, the redness disappears, and the ulcers cicatrise or heal. The favourable results in the case of lupus have naturally led to the extension of such photo-therapeutic methods to other diseases, whilst experiments have also been made with the Röntgen rays and radio-active substances.

The clinical results that are at the present moment being claimed for photo-therapy are numerous, but accurate experimental data are unfortunately scanty. Hasty

¹ There is now some doubt as to the efficacy of the red-light treatment of small-pox.—ED.

generalisations based on an imperfect knowledge of a given agent and its mode of action tend to discredit an otherwise promising line of inquiry. The unicellular organisms are particularly adapted for the purpose of testing the scientific basis of light therapy, as well as for advancing its boundaries in legitimate directions. A recent result in this direction has been the exact determination of the bactericidal lines of the spectrum.

LECTURE II.

Dominant Line of Research is the Physiology of the Cell—
Significance of Micro-Organisms in the Economy of Nature—
Nitrification—Phosphorescence—Bacteriology and Hygiene.

In our opening lecture we stated that the dominant line of research at the present time was with reference to the physiology of the cell. The study of the cell occupies the forefront of biological investigation. Whatever secrets it may still have to reveal, the cell, so far as our present methods of analysis reach, is the unit of life—viz. a nucleated speck of protoplasm, whether one regards the animal or the plant cell. The connection between animal and vegetable is being brought closer, and the cell, whether plant or animal, exhibits all the essential vital phenomena. And the main expressions of active life are in their essentials identical in plant and animal cell. In other words, as regards essentials in both the plant and animal kingdom, the structural and functional units are identical. The higher organisms are colonies or republics of cells, and a cellular structure forms the basis of all normal vital processes, whether simple or complex. This conception forms the connecting link between the various branches of biological inquiry. Every physiological and every pathological problem is, therefore, ultimately a cellular one. We noted the influence of this conception

on modern research, and the increasing concentration of inquiry and of experimental methods upon the cell. One of the results has been, as indicated in the last lecture, the revelation and inclusion within our powers of observation of a teeming world of animal and plant life lying beyond the ordinary range of human vision—a world far exceeding in numbers the higher plant and animal life on the surface of the earth. Of these forms the bacteria and protozoa are typical. They constitute a microcosmos, and reveal how great Nature can be in small things. In these simple forms entirely devoid of organs and tissues the essential processes of life are as completely carried on as in the complex and higher forms of being. We gave certain instances in the first lecture of the results obtained by recent methods of research upon these lowly but most important forms of life, *e.g.* the protozoa and the bacteria.

We have spoken much of the cell. The cell, of course, is a microscopic object. For ordinary unaided vision the lowest standard of measurement is the millimetre ($\frac{1}{25}$ inch). For microscopic objects, such as the cell, the standard of measurement is the $\frac{1}{1000}$ part of a millimetre, or a *mikron*. The human blood cell has a diameter of 8 mikrons, whilst that of a bacterium is still less, being about 1–2 mikrons. In physics and chemistry the measurements are still finer—the mikron is further subdivided into $\frac{1}{1000}$ parts, and molecules, &c., are theoretically measured in $\frac{1}{1000}$ parts of a mikron. The microscope is, therefore, indispensable in the study of the cell, as we have already indicated. In this direction a great elaboration and refinement of technique has been

brought about in addition to the evolution of staining methods.

Our survey would be incomplete without special reference to recent results obtained with regard to the significance of micro-organisms in the economy of Nature. The yeasts have been closely studied, and pure cultures employed with success in the brewing industry. The moulds have been studied likewise, types of which are familiar on jams and in green cheeses. In many alcoholic processes they play a part, notably in the symbiotic fermentations as unconsciously used in the East (p. 195). The moulds in many instances produce diastatic ferments, thereby saccharifying starches and preparing the way for the alcoholic yeasts, and in this way koji, saké, and other Eastern beverages are prepared (p. 195), whilst in certain diseases, such as ringworm, they appear as human parasites.

We have already referred to the action of bacteria in resolving dead organic matter into assimilable forms of food for plants and animals. The overwhelming majority perform beneficial functions in the economy of Nature. They institute a number of processes essential to the self-purification of water and soil, and to the everyday processes of agriculture. All the higher forms of life really depend for their existence and well-being on the never-ceasing activity of these humble forms of life in Nature. One instance may be given, as it is at the same time amongst the most recent additions to our knowledge. Agriculture requires not merely seed and soil, but a stock of the proper kind of bacteria; the soil bacteria are vital factors in the food cycle of Nature. Two essentials for

plant food are carbon and nitrogen. The main sources are the air for carbon and the soil for nitrogen. The former is taken up by the plant as carbonic acid, but the nitrogen comes from the soil, where it exists as nitrates, which the plant builds up into proteids. The nitrogenous waste products of animal life are not lost ; they are converted into utilisable nitrates by plant cells. The process is termed nitrification, and the agents are bacteria. The nitrogen is raised through the stage of nitric acid (by oxidising processes) to a union with soil bases to form nitrates, or the best fertilisers for farm crops. It is a biological process due to the action of bacteria, and it is of a twofold character. Nitrites are first formed, and then nitrates, by bacterial action, and the organisms engaged are termed the nitrous and nitric bacteria. When both kinds are present the ammonia becomes completely oxidised to nitrates. These organisms are widely distributed in soil, and they are of great importance, as they deal with the mineral world and render the elements of the same assimilable by the plant and render soil fertile for plant life. Their study is most important for agricultural processes ; indeed, agriculture, like brewing, is tending to become a scientific process.

A number of luminous objects occur in Nature, the light being due to the activity of lower forms of animal and vegetable life. The sea phosphorescence, due to such minute animal forms as *Noctiluca*, is familiar to all ; and in the case of dead fish phosphorescence is frequently observed. This is due to bacteria which multiply on the fish and emit light, and the phenomenon is the most perfect example of light without heat. The phosphorescence

is a phase in the vital activity of these unicellular forms of life, and appears to consist in oxidation processes occurring with the cell. It is essentially a vital process, as it ceases with the death of the organisms. A number of bacteria possessing luminous properties have been isolated, and their luminous properties are evinced as readily in the laboratory as in Nature. If we were able to reproduce such effects we should be in possession of the ideal illuminant—*i.e.* a body capable of producing light without heat. Again, the parasitism of bacteria in the living bodies of man and animals brings with it a number of disease processes. The study of bacteria as disease agents constitutes the important chapter of medical bacteriology, and in recent times diseases such as tuberculosis, typhoid fever, cholera, plague, diphtheria, etc., have definitely been associated with an invasion of specific micro-parasites belonging to the class of bacteria. Their study has brought about a reconstruction of modern hygiene. For example, in epidemiology, a knowledge of the exact agents at work enables us to take better measures for the prevention of the spread of disease; whilst disinfection is now more accurate, as the dosage of each disinfectant necessary to kill a specific disease agent can be experimentally determined in the laboratory. In this way the relative value of disinfectants can be worked out, and this is of great practical value. The hygienic conditions of water, air, and soil can now be more accurately studied as harbouring germs of disease, and the bacteriological examination of water gives many clues as to dangers or as to its general potability, whilst biological methods are now being employed in sewage

processes, and methods based on the sanative action of bacteria have recently been introduced.

There are still gaps in our knowledge—in rabies, small-pox, scarlet fever, measles, cancer, and many others. The essential causes of these diseases are unknown to us at present.

New methods are required for a fresh study of the unknown factors of disease. They may be beyond the range of microscopic vision; our culture media and methods may not be suitable for their detection. There may be mixed infections in which one organism prepares the soil for another. There may be organisms which pass through the pores of the Chamberland filter. It appears to me we are at a turning-point where the want is pressingly felt of fresh methods of work. In any case, disease infection appears to demand some agent capable of self increase and multiplication.

The cultivation of protozoa is at present impossible on ordinary culture media,¹ although their parasitic properties have been demonstrated. They are strictly obligatory parasites and require living hosts. Of protozoan infections a classical example is malaria; also tsetse-fly disease and numerous infections of lower animals. Recently a protozoon has been found in sleeping sickness of Africa (fig. 12).



FIG. 12.—The *Trypanosoma gambiense*, the protozoan parasite of sleeping sickness ($\times 3,000$).

¹ A few protozoa, such as amœbæ and certain trypanosomes, have been cultivated on artificial media since this was written.—ED.

If this be so, we may expect in the future great advance in preventive measures against this disease.¹

Few of the lower animals are free from such protozoan parasites; they are common, for example, in rats, reptiles, fish, and birds. In many instances they appear to do little harm; in others their action is as deadly as in malaria. They may occur intercellularly—*e.g.* in the blood vessels, glands, ducts, and between muscle fibres; also intracellularly, as in epithelial cells and in blood cells (malaria). Few lower animals are free from protozoan invasions of some kind or other, and this has influenced medical research, as, for example, with reference to cancer, dysentery, and small-pox. Much, however, is as yet theoretical, and the interpretation of observations varies much.

The Sporozoa are the most important group of such protozoan parasites. Intracellular parasites are particularly deadly because they grow at the expense of the cell—*e.g.* in fishes, forming ulcers and tumours—and a group called the Coccidia are found in nearly all the tissues of the lower vertebrates. A typical example is the coccidial disease of the rabbit, which attacks young rabbits when they commence to eat green food, and produces a wasting away and death. The parasites settle in the intestines, the bile ducts, and the liver, and become attached to the epithelial cells in those situations. They have a complicated life history, and this generally renders the study of protozoan infections both toilsome and difficult. In the coccidial disease a capsulated form is

¹ A trypanosome (*T. gambiense*) seems undoubtedly to be the cause of sleeping sickness, and an important International Commission has recently formulated various measures for the control of the disease.—ED.

swallowed, the capsule becomes dissolved by the digestive juices, and amœboid forms result, and enter upon a sporulating stage, in which they attack the epithelial cells of the intestine, and a fresh development of the fully formed parasite results.

Pébrine, a disease of silkworms, is also due it would appear to a protozoan infection, which was studied by Pasteur.

In malaria likewise complex phases in the life history of the parasite occur. Thus spherical amœboid bodies develop in the blood cells, and live at the expense of, and destroy, the cells (fig. 13). Flagellated forms, crescentic bodies, and sporing forms or rosette bodies are also met with. The parasite cannot be cultivated outside the body, but persons inoculated with diseased blood contract malaria. The life cycle is a complicated one, and includes a development from free spore to intracellular parasite and at certain stages the production of sexual elements. The blood is the main seat of the infection, though in malignant cases the parasites are numerous in the internal organs, *e.g.* the brain and spleen. There are essential differences in the time of sporulation which appear to determine the various types of fever. Thus we have the quartan fever, in which a period of seventy-two hours or thereabouts elapses between the commencement of one attack of fever and that of the next attack; the tertian, in which forty-eight hours similarly elapse; and the quotidian,



FIG. 13.—Malaria parasite in a red-blood corpuscle ($\times 3,000$).

in which only twenty-four hours elapse. Quinine seems to be a poison to the parasites, and causes their disappearance. Cognate blood parasites are also met with in the bird, frog, lizard, tortoise and other amphibians, and these are at present being closely studied.

The malaria parasite is conveyed to man by the bite of the mosquito. The parasite therefore exists in two separate phases or stages—as a parasite in man, who acts as an intermediate host and in whom the cycle of its development produces the symptoms of malaria, and as an extracorporeal parasite which lives and develops in the body of the mosquito, which is its definitive host; that is, a host in which a sexual cycle of reproduction occurs. You will understand, therefore, the war that is at present being waged against the mosquito to lessen the danger of infection in the human subject. Mosquitoes belonging to a certain group, the Anophelinæ, act as the definitive hosts, and appear to be the only channel by which infection is conveyed from man to man. The campaign is therefore against this anopheline group of mosquitoes, which is to be met with in all malarial districts. The majority of biting midges and mosquitoes appear to be incapable of acting as hosts to the malarial parasite, whilst all malarial districts contain anophelines. So far as is known the parasite only exists in the mosquito and in man. The mosquito is a country insect, and lays eggs in small shallow puddles or slow streams. The parasites find their way into the salivary glands of the mosquito, are inoculated thus into man by the bite, and develop in his blood into fresh young parasites.

This will help us to understand the modern prophylaxis in malaria, which consists in providing houses with wire-

gauze screens and mosquito-nets to keep away the insects, a direct campaign against the mosquito, drainage of breeding places, and the application of petroleum to such collections of water as cannot be drained in order to prevent development of the larvæ, and quinine as a parasiticide, for the daily or weekly use of quinine largely prevents the development of the parasites in man.

The study of the protozoa, it will be seen, is difficult. The bacteria have a simple life history, a simple multiplication, a direct invasion of the body, and they can, as a rule, be cultivated in the laboratory. The protozoa, on the other hand, have a complicated life history, and we cannot cultivate them. Their cycles of development may include various hosts amongst man and the lower animals; all the links have therefore to be traced in understanding and combating effectively such disease agents. A comparative parasitology is being slowly evolved, and no doubt the time will come when this study will result in the discovery of parasiticides which may be employed in the direct treatment of such diseases.

The protozoan infections are largely transmitted by insects—for example, the tsetse fly disease of horses, conveyed by the tsetse fly. The discovery of a trypanosome recently in sleeping sickness would point to insect infection, and at present search is being made for a culprit insect, which will probably be successful.¹

One point recent research has brought out very clearly—the importance as factors in the transmission of disease to man of the lower animals, such as insects, fleas, and

¹ The trypanosome of sleeping sickness appears to be conveyed by a tsetse fly, the *Glossina palpalis*, and perhaps by other biting flies.—ED.

rats. In our campaign against disease we have therefore to consider in many instances not merely the individuals affected, but the living links in Nature which may harbour and transmit infective agents to mankind.

There is therefore a general tendency at present, as you will have seen, to bring the problems of transmissible disease within the fruitful range of laboratory study.

LECTURE III.

Manner in which Disease Organisms Produce their Effects—
Toxins—Antitoxin Treatment—Agglutination—Life at Low
Temperatures — Intracellular Processes — Cell Ferments—
Methods of Investigation.

We have referred to the appreciation that now exists of the significance of micro-organisms in the economy of Nature. The overwhelming majority of these minute forms of life discharge beneficial functions, and it is only a relatively infinitesimal number which have the power of producing disease. With regard to the latter, the most important parasites belong to the lower members of the vegetable and animal kingdom. The vegetable parasitic forms constitute the main chapter of study in present day bacteriology, viz. the infective bacterial diseases such as are due to the invasion of the system by bacteria—for example, tuberculosis, diphtheria, and typhoid fever, &c. The animal parasites are the subject-matter of animal parasitology or protozoology, in which diseases are due to minute animal parasites, notably protozoa, *e.g.* malaria, tsetse fly disease, &c.

The cultivation of the bacteria renders their study easy and enables us to get nearer a solution of the problem of how they act. In what do their virulent properties consist? Recent research has thrown much light on this

subject; and the conclusion now is a pretty general one that the bacteria act in virtue of poisons which they produce in the course of their multiplication in the body. This has been demonstrated in the case of diphtheria and of tetanus. Every infection would appear to result in an intoxication due to poisonous bacterial products. In diphtheria and tetanus death is due, not to the infection—the mere presence and multiplication of the invading bacteria—but to poisons acting on the centres of life, and notably the nervous system. The organisms grown in the laboratory produce soluble toxins, which, apart from the organisms, are capable of producing the essential symptoms of the above diseases, viz. the intoxication. These diseases are thus essentially intoxications. The broad fact has been established that it is therefore not so much the infection as the intoxication that has to be combated—in other words, an antitoxic treatment of these diseases is the rational therapeutics. Can antidotes or antitoxins be prepared? This has been successfully done in the case of diphtheria, the toxins of which inoculated into a suitable animal, such as the horse, result in the elaboration in its system of anti-bodies or antitoxins to the poison in question. The blood serum of the treated animal laden with these antitoxins introduced into the system neutralises the poisons produced in the natural course of the disease. By this treatment the case-mortality in diphtheria has been reduced from 28–30 per cent. to 8–10 per cent. This is a typical example of what is essentially meant by antitoxin treatment of disease, or, as it is sometimes termed, serum therapeutics.

In many instances the disease germ does not seem to produce excreted soluble poisons, but there is good reason to suppose that toxins do exist, though they are not extra- but are intra- cellular, *i.e.* are present within the bacterial cell, just as the alcoholic ferment is present within the yeast cell, *i.e.* there may be toxases analogous to zymase. One point must be referred to, *viz.* certain profound specific properties of cells revealed by recent study. A typical instance is the phenomenon of agglutination—a specific reaction induced in the body of the individual by different invading organisms, *e.g.* that of enteric fever, whereby the blood serum of the patient induces a clumping or agglutination of the infecting bacterium. The blood of the enteric fever patient immobilises and clumps the bacilli of typhoid fever. It has become a specific test of practical value in the diagnosis of this and other infective diseases, *e.g.* the micro-coccus of Mediterranean fever is agglutinated by the blood of a person suffering from the disease, *viz.* Mediterranean fever. In this way obscure cases can frequently be diagnosed by the medical man with a certainty previously unattainable. A differentiation is thus rendered possible between malaria, Mediterranean fever, and enteric fever, cases of any of which sometimes resemble one another.

We may finally refer to one or two further salient features of recent biological research, preferably those of general rather than of specialised biological interest. Life is dependent on certain physical conditions, and notably upon temperature. The active processes of life cannot take place above or below a certain degree of temperature. The maximum and minimum temperatures

compatible with vital activity vary for different forms of life. Man, in virtue of the heat-regulating mechanism of his body, is able to tolerate great variations in external temperature. This is not the case with most other forms of life. If we regard living organisms generally, we find that temperature requirements are not uniform, but are rather of the nature of a sliding scale. In this respect the unicellular organisms, the bacteria, exhibit a remarkable range extending from zero to 70° C. There is, however, for all living cells, free or united, a thermal death point at which life is destroyed. It has been found that cells are much more susceptible to the influence of heat than of cold. Extremes of heat and cold suspend the vital activities of living organisms, but their permanent destruction is more readily accomplished by heat than by cold. Hence the practical success of methods of heat sterilisation, *e.g.* as regards the infective agents of disease in our surroundings. Heat is the most complete and certain form of disinfection. Recent research has shown that whilst a marked reduction in temperature will inhibit the activities of life, it will not necessarily destroy life itself. For example, bacteria exposed to the arctic temperature of liquid air (-180° C.) pass through the ordeal without loss of life. Even an exposure of six months at this temperature does not succeed in robbing the cells of their vitality. And a further plunge to the temperature of liquid hydrogen produced no apparent deleterious effect. With the lowest temperature at our command the limits of vitality have not been reached. These results are of considerable interest to a speculative mind. It is feasible to assume that at such temperatures

the living substance is frozen to ice and that the ordinary chemical processes of the cell cease. Yet life is principled even in a dormant condition. What, then, is the exact basis of life? Is it of a physical rather than a purely chemical nature? Are we in a position to stop the chemical processes without affecting the living principle itself? If so, the theoretical bearings of the fact would be of the greatest significance as regards the ultimate problem of vitality. In this connection the survival of life after six months' exposure to -180° C. is significant; there must have been a state, as it were, of suspended animation. To emphasise our view of the ranges of temperature that some unicellular organisms tolerate, it may be stated that organisms live at temperatures extending from 70° above to 200° below zero on the Centigrade scale. The results appear equally to favour the possibility of the extra-terrestrial origin of life and its possible conveyance to the earth by an aerolite or cosmic dust.

The last and perhaps most important reference to recent methods and results is with regard to direct investigation of the life unit, the cell, and notably with regard to the processes which occur *within* the cell. These form the fundamental basis of the cell's vital activity. Their study means a more intimate knowledge of life itself as distinguished from its outward activities—a study not merely of function, but of the processes on which it rests. The inherent difficulties of such investigations are very great. The ordinary methods of studying cell life are of a chemical and a physical nature, and some of these were referred to in the first lecture (p. 244). The methods of

this character are mainly applied to dead matter, which no longer represents or possesses the qualities of the living tissues. The heating, fixing, and staining methods of ordinary microscopical research essentially modify the living substance. The material examined is degraded and modified living substance. Observations under these conditions yield valuable information as to the physical framework, but do not necessarily give a clue as to the functions or the basis upon which the functions rest. Paradoxical as it may appear, the stability of vital phenomena rests on the instability of their physical basis, which is at every moment undergoing change. The difficulty of study as regards the living cell is therefore apparent. At the same time, the ordinary methods do not favour the attempts made to solve the problems. One great aim of physiological inquiry must therefore be to attempt to get somewhat closer to the study of life and its processes than has hitherto been attained. For this purpose the usual methods of research are inadequate, and particularly with regard to the essential processes of life as they occur within the confines of each cell, when it comes to the direct study of the cell; the most likely course appears to be by mechanical methods of attack, *i.e.* methods which, whilst bringing the cell constituents within the field of study, will at the same time interfere with and modify as little as possible the sensitive cell material with which we are dealing. To do this we must rupture the cell and extract the cell plasma, and in the process obtain the cell plasma as unchanged as possible. Why is this? Because we wish to investigate not the structural but the chemical organisation of the cell. In active life chemical

processes are continually going on, and a transformation of chemical into other forms of energy, and *vice versa*. These vital cellular processes occur after the absorption of the food and before the excretion of waste products, *i.e.* they are localised and centred in the cell itself. These fundamental processes are not discoverable by the ordinary methods of inquiry. The knowledge we possess is mainly of the secretory and excretory processes of the cell and processes of dissolution, rather than the building-up processes. In short, what ordinary chemical research has been able to study hitherto, has been almost entirely matter on the down grade, and passing back to the stable condition of the chemical bodies as they exist in the outside world. The processes associated with ordinary digestion and secretion have been closely studied outside the cell, and the activities of the soluble cellular ferments critically investigated. Recent research has shown that the cell-plasma likewise contains peculiar ferments of its own, *i.e.* intracellular ferments; in fact, the cell is not so simple as it looks, but is highly organised in structure and in function. The intracellular processes are varied and complex. The complexity may be illustrated by ordinary functions of the cells of such an organ as the liver. It forms glycogen from sugar, breaks up blood pigment, forms bile pigment, secretes bile, neutralises many poisons, and conducts a number of additional fermentative processes, and these take place in the liver cell—about as large as the one-thousandth part of the head of a pin—and the functions are all based on intracellular processes of a chemical nature. What are the subtle reagents which the cell has at its disposal? They are of

the nature of ferments. It is these intracellular agents the study of which is at present so important and significant for an understanding of cell life. We have already mentioned how in simple organisms—*e.g.* *Amœbæ*—digestive processes are intracellular. These ferments exist in plant and animal; they appear to be the tools of the cell. Take the liver; it seems to contain proteolytic ferment, maltase, nuclein-splitting ferment, aldehydase, fibrin ferment, and probably a lipase and a rennet-like ferment; and such organic cells produce antihæmolysins, antitoxins, etc. The cell when it dies becomes a prey to its own enzymes, and a rapid auto-digestion occurs—a formation of simpler and more diffusible substances. We have thus a liquefaction and non-bacterial decomposition of the organs, as they become the prey of their own ferments and are no longer under the restraint of the conserving vital forces. What are the factors which direct and regulate these wonderful arrangements during life and ensure the cell automatism? We know that there exist pre-ferments or zymogens, which are only rendered functionally active in the presence of certain substances and act in certain directions—*e.g.* the pepsins. They are actuated under the influence of certain stimuli, and one stimulus may lead to a certain form of activity, which in its turn induces another process, and thus a complicated and interdependent series of events may occur, all essentially linked together, and of which we only see or can grasp the end links of the chain.

In addition to a structural and functional the cell must have a chemical organisation as well, as is borne out by the above facts. There is a colloidal substratum

which not merely functionates but elaborates the necessary agents for its internal metabolism. And these are probably of an enzyme-like nature and constitute fermentative processes. The term 'fermentation' was first applied to the oldest studied, the alcoholic, whereby alcohol and carbonic acid are formed out of fermentable sugars. It was proved to be a biological process associated with living cellular agents, the yeast cells. Similarly, many other fermentations are now definitely associated with living agents. Fermentation has therefore become permanently a biological study, and enzymology must be made a special branch of physiological inquiry. One of the most widely significant researches in the biological field has been carried out by Buchner on the yeast cell. By mechanically triturating the yeast cell he was able to obtain its cell plasma, free from living and intact yeast cells, and found that this plasma or 'zymase' was capable of fermenting sugars into alcohol and carbonic acid. He thus demonstrated a cell-free alcoholic fermentation, and that the cell, deprived of its organisation, was capable of producing the fermentation in virtue of the chemical activities residing in its plasma. It was an intracellular process of a fermentative character, most valuable for biological research generally, and an indication of the great possibilities in the biological field as a whole. This has given a great impetus to intracellular study. A whole field of endocellular bodies with active chemical properties to be studied. Research in this direction has been extended to the higher organisms with utilisation of analogous methods for obtaining cell juices of organs. A development of mechanical methods has resulted, and this is undoubtedly the right way of

attack, notably in the case of the fresh organs and tissues of the animal body. The trituration is best accomplished by

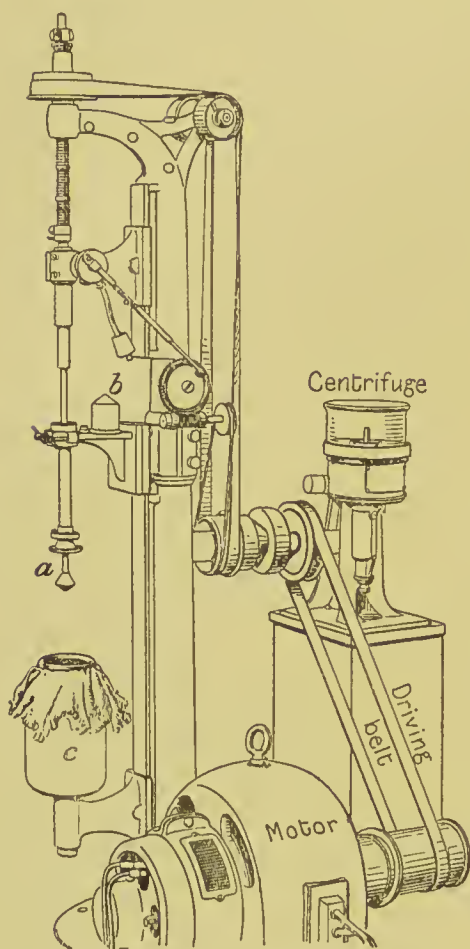


FIG. 14.—Diagram of the machine employed by Dr. Macfadyen for tritulating bacteria. *a* is a cone revolving at a high speed in the pot *b* (here detached), in which the bacterial paste to be ground is placed. This is cooled in the vessel of liquid air, *c*, in which it is immersed. The machine is driven by the motor. The centrifuge is for separating the bacterial debris from the bacterial cell-juice after grinding.

cold grinding methods to secure absence of alterations during the grinding process. For such lines of investigation the following points are to be regarded as essential to success : (1) Fresh tissues or cells ; (2) immediate manipulation on account of auto-digestion ; (3) a good and perfected mechanical process ; (4) avoidance of heat development and changes during the grinding process, *e.g.* cooling by water, brine, or carbonic acid ; (5) a quick and immediate manipulation of the juices. The spleen has been studied in this way and proteolytic enzymes isolated from it, and the remarkable fact elucidated that it contains not only a pro-

teolytic enzyme which acts in an alkaline but one also which acts in an acid medium : *i.e.* it gives products of

alkaline pancreatic digestion in an acid medium. These results are new and of great importance. The study of intracellular enzymes will yield profounder knowledge of processes of the body not only in health, but likewise in disease. These investigations with such methods are being rightly pushed at present.

It was mentioned that bacteria retain life at the temperature of liquid air. It has likewise been found that at this low temperature these cells become extremely brittle, and suggested the possibility of triturating them at this low temperature. This has been successfully accomplished without any admixture of sand and kieselguhr (fig. 14). The frozen cells can be completely disintegrated, and the cell plasma of these minute objects ($\frac{1}{1000}$ part of a millimetre) obtained. The typhoid bacillus has been proved to contain within itself a toxin; and in this fashion it has been rendered possible to obtain the devitalised intracellular toxins of a number of bacteria. These introduced into animals have been found, for example, in the case of typhoid to have valuable immunising properties, and to produce antitoxic and bactericidal serums.¹ This has given a fresh impetus to study of bacterial toxins and their properties, and may have important bearings on the preparation of vaccines and antitoxins in treatment of many diseases, as well as a fresh study of cellular factors in immunity, and of the cell (as an immunising agent)

¹ Dr. Macfadyen at the time of his death had just completed important researches on the intracellular toxins of such organisms as the typhoid bacillus, pneumo-coccus, cholera vibrio, &c. He showed that the intracellular juices of these organisms are markedly toxic, and on injection into the horse produce anti-sera possessing immunising and curative properties. In this way he anticipated that therapeutic sera might be prepared for the treatment of the diseases caused by these micro-organisms.—ED.

per se apart from its products, investigations more precise and results more accurate than have previously been obtained.

The twentieth century has to establish a Cellular Physiology; and I feel sure that by these methods a new and fruitful era in biological research has been initiated as regards unsolved problems by the endeavour rightly not to fit the problem to the method, but the methods to the problem.

TOXINS AND ANTITOXINS

A COURSE OF FOUR LECTURES DELIVERED AT THE ROYAL
INSTITUTION, FEBRUARY AND MARCH, 1899

LECTURE I.

Cellular Structure of all Organisms—Interdependence of Cells—
The Bacteria—Bacterial Toxins—Disease-producing Bacteria
and their Mode of Action—Bacterial Poisons.

In the course of lectures that I shall have the honour of delivering at the Royal Institution, it is my intention to deal with the subject of toxins and anti-toxins. In our consideration of this subject it will be necessary to touch upon matters that occupy the forefront of bacteriological inquiry at the present day—matters, in fact, that bring us to the frontier line of our present knowledge. In many respects we are still on the threshold in this difficult branch of inquiry, and much yet remains for painstaking research to accomplish. But if we consider that Bacteriology, as a concrete branch of inquiry, has been a development of our own times, and that it has meant the opening up of a new world—a world of the infinitely little—to our gaze, the results of the past few years will appear rich and varied, and they have already thrown a flood of light on the conditions that make for health and for disease. It will be my endeavour to place some of these results before you, and I am encouraged to do so by the fact that we are not dealing with a subject of purely academic interest or of abstract research. The questions involved

are questions that have a deep and permanent interest for each one of us, inasmuch as they deal with the origin and prevention of suffering and disease.

I would ask your consideration for a short time, whilst I briefly place before you certain facts that form the basis, and that have smoothed the way for successful research in this branch of inquiry—facts that will give us a clearer understanding of the special subject-matter of these lectures, and that form landmarks in the development of modern Bacteriology. If we take the human frame we find that the phenomena of life depend upon the harmonious working of certain organs, whether of digestion, respiration, or circulation. The normal activity of an individual's life depends upon the successful action of these various organs; their harmonious action means health, a disturbance of the same means ill-health. These various organs may be compared to a federation of republics, each one being ruled by its own laws adapted to the special functions it has to fulfil. If we consider these organs or republics more closely we find that they are built up of tissues, ministering to given aims in the economy of the body. But as a republic consists of many citizens, so we find on closer examination that an organ or its tissue is made up of many individual elements. These individual elements are known as cells. The cell is the unit—it is the structural unit, it is the functional unit. All the conditions for the essential functions of life are present in the cell, and hence it has been called by some the elementary organism. The cell has, however, been fully considered in previous lectures contained in this volume, and need not now detain us.

Perfect, absolute health would imply all organs, their tissues and cellular elements, being structurally and functionally normal. In this sense I am afraid that no one can boast of health. Health, as a matter of fact, passes over with many gradations to distinct and appreciable disease; it is therefore evident that health and disease are relative terms. But any departure from health, however slight or from whatever cause, is primarily a *local* one, *i.e.* it affects single cells or groups of cells. The fact that the cells of the tissues are connected together, furnishes the possibility of the spread of a disease process from cell to cell in one and the same organ, whilst a diseased cell by transference to another part of the body may carry the morbid process to other organs. And, on the other hand, the blood and lymph that bathe all the tissues of the body may transfer locally-produced injurious agents which may cause a general and fatal disturbance of the whole system.

If the normal functions of life are to be understood in their fundamental phases it must be by a consideration of the living unit—the cell; and similarly morbid processes are only understood by a study of the diseased cell. The study of structure precedes that of function. Anatomy, formerly structural, has now passed on in histology to the intimate study of the cell. Botany is becoming devoted more and more to the observation of the plant cell; and Pathology is now largely a cellular science. We are being driven more and more into the laboratory to elucidate with the aid of the microscope and other means the deeper secrets of nature. Infection and disease represent a struggle between cells, and noxious products, the result of cell life,

act upon healthy cells and thereby destroy or pervert their functions. It is evident that disease is not to be understood by a mechanical interpretation, but that its study in the highest sense is a biological one.

Health and disease are, then, primarily the results of cellular processes occurring within the body. But besides these internal conditions, the human frame also relies for health upon a large world outside itself, and the influences of this outside world are of a many-sided nature. The food we take is in large measure provided by the lower animals, and if diseased may injuriously affect us. The lower animals in their turn depend largely on the vegetable kingdom for the support of their life processes. And the members of the vegetable kingdom depend on the elements of the soil and the air for the matter that sustains them. In short, there is a perpetual and beneficent circulation of matter in the world, and amongst the most important factors that aid this circulation are minute, cellular forms of life—the micro-organisms or microbes. The great store-house in Nature for these minute forms of life is the soil; there these ubiquitous forms are ever at work in the great redistribution of the dead matter, whether animal or vegetable, that finds its way to the soil. Through the splitting up of dead organic matter and the restoration of its elements to the soil and air, these elements are enabled to re-enter the circle of life. Life, indeed, would become impossible were it not for the action of bacteria in cleansing out Nature's dustbin. The majority of micro-organisms play an important part in the preservation of life and health, whilst, fortunately, an infinitely smaller number are known to us as causes of

disease. This large group of unicellular organisms belongs to the lowest rank of the vegetable kingdom, and these living forms are known as micro-organisms, bacteria, or microbes. The individual forms cannot be detected by the naked eye—they can only be studied with the aid of the best lenses; it is a living world that has been revealed to us by the microscope. The organisms that concern us in these lectures are bacteria—so called on account of their frequent rod shape—and their study is the science of Bacteriology. For the medical investigator who is not a botanist the word ‘bacteria’ does not imply a group sharply and scientifically defined. Under this name we are in the habit of grouping all those microscopic vegetable cells which by their influence are capable of affecting the health of man and animals. These organisms present various types of form, and on their external peculiarities a crude classification has been built. We thus speak of spherical, rod-shaped, and curved forms—known respectively as coccus, bacillus, and spirillum—and familiarly compared to a billiard ball, a lead pencil, and a corkscrew (see plate, p. 42). Amongst their main characteristics we note the enormous rapidity of their reproduction, which is accomplished in the simple fashion of fission or cell division: hence the name sometimes applied of ‘Fission Fungi’; and in this great rapidity of multiplication there is a compensation for their small size. I may state, in illustration of this, that *one* anthrax bacillus when introduced into the blood is capable of reproducing, as regards numbers, the population of London in a very few hours! We are also struck by the varied conditions under which these organisms can live—

viz. from freezing-point up to temperatures of 60° and even 70° Centigrade, *i.e.* extremes of temperature at which the functions of ordinary protoplasm are destroyed (see p. 367). Further, we should note that the bacteria are able to sustain themselves on simple or complex forms of food, and recognise the property they possess of living as saprophytes on dead organic matter, or of multiplying as parasites at the expense of the animal body. Our more accurate knowledge of these organisms is, as I have already indicated, an achievement of comparatively recent times. At first those who studied them were principally botanists, and we must remember that bacteriology was originally a branch of botany, and in its study we recognise the help that has been afforded by the work of Cohn, De Bary, Nägeli, Zopf, and Brefeld, who have done so much to forward our knowledge of micro-organisms as vegetative forms. This help is still required, just as in the study of the protozoan agents of disease the aid of the zoologist is essential. We follow with deep interest the work of such men as Bütschli and Fischer, who are endeavouring to penetrate into the mysteries of these cells and to reveal structure in what we were wont to describe as structureless organisms, the bacterial cell appearing to ordinary observation as homogeneous protoplasm surrounded by a containing wall or membrane.

The history of bacteriology has already been detailed in previous lectures contained in this volume, and need not be further alluded to.

What do micro-organisms accomplish in their varied work? The *yeasts* bring about various fermentations, notably the alcoholic.

The *bacteria* are engaged in the sanative processes of a fermentative and putrefactive character in Nature. In soil they also oxidise ammonia to nitrites and nitrates, which are utilised by the plant world, as Winogradsky's important observations have proved. They produce acetic acid or vinegar, they can ferment sugars, they ripen cheese and spoil milk. Various fermentations of technical importance are due to their action—*e.g.* the tobacco fermentation. From what I have said you will gather that the study of bacteriology has a wide significance outside medicine and pathology.

Enzyme action can be demonstrated in a medium from which after growth the organisms have been removed (see p. 170); these enzymes are therefore of a soluble nature and pass out from the cells. As we shall see, the action of the soluble products of the bacterial cell is of significance in disease processes. Among the products of this cell life we shall have to consider in these lectures such as are of a poisonous nature, and have hence been termed *toxins*.

It is an old belief that infectious diseases are due to a living contagion. Pollender and Davaine noted the presence of minute rods in the blood of anthrax animals, and in the sixties observation was made of the presence of organisms in pyæmia and blood-poisoning. Pasteur's study of chicken cholera and other affections, and Lister's work, had a wide-reaching influence in stimulating bacteriological research upon disease. A German surgeon once, in expressing to me the effect that Lister's teaching had, said: 'We used to wash our hands *after* the operation, now we do it *before*.' Anthrax or splenic fever is a

classical instance of the results obtained by means of Koch's methods. The rods or bacilli found in the blood of affected animals can be cultivated for many generations away from the body of their host and still remain capable of producing the disease. In a freshly infected animal the same organism is found and can be re-isolated from its blood. The causal relation of the living infective agent to the disease was thus firmly proved, in the same way as the causal relation of certain organisms to the putrefactive and fermentative changes that occur in dead matter. The cardinal fact was demonstrated that infectious diseases are due to the action of living cellular agents, and that the majority of those isolated belonged to the group of the bacteria. To produce an infection it is necessary that the given organism should be capable of living and multiplying in the tissues of the body. Many are not able to do so; these are the 'saprophytic' forms, while those that can do so are described as 'parasites.' Of these, some, such as the tubercle bacillus, appear capable of existing only in a living host, and are therefore transmitted by living links consisting of either human beings or animals. Others, such as the anthrax bacillus, appear capable of leading a vegetative life in the soil and a parasitic existence when opportunity offers. In such cases infection may take place through any matter that happens to contain the organism. The saprophytic bacteria may, however, injuriously affect the body, without being present in its tissues, by the production of poisons in our food. In this instance the morbid effect is not described as an infection, but as an intoxication, *i.e.* a poisoning through poisonous products.

Among the infectious diseases of which Koch's methods have enabled us to detect the living cause we may mention :—

1. *Enteric*, or *typhoid*, *fever*, caused by a bacillus.
2. *Cholera Asiatica*, due to the 'comma' bacillus.
3. *Tuberculosis* and *consumption*, due to a bacillus.

One of the greatest discoveries of the nineteenth century was that this scourge of the human race is an infectious and a *preventable* disease.

4. *Leprosy*, caused by a bacillus.
5. *Diphtheria*, caused by a bacillus.
6. *Influenza*, caused by a bacillus.
7. *Tetanus*, caused by a bacillus.
8. *Glanders*. 9. *Anthrax*. 10. *Plague*. All due to bacilli.
11. Forms of *Pneumonia*.
12. *Inflammation* and *Suppuration*, due to cocci and other forms of bacteria.

The discovery of a specific agent in a disease and the study of its action and life history hold out the promise of preventive treatment, and the greater part of modern hygiene has been built upon the discovery and study of the infective organisms I have just mentioned.

We have now to ask ourselves what occurs when one of these infective organisms gains access to the body. In this respect we note differences, which I shall endeavour to explain briefly. I mentioned at the beginning of the lecture that every infection is in the first instance a local one; by infection meaning the attack upon the living cells of the body by another living cell or micro-organism. And the parasitic bacteria may remain at the local seat of

infection, and multiply there without spreading generally through the system ; thus, the organisms of suppuration may remain localised, as in the case of an abscess or boil. We have one of the most striking examples in the tetanus (lockjaw) bacillus ; if this bacillus gains access through a wound, it remains and multiplies at the point of entrance. In diphtheria we also find that there is a site of multiplication of the diphtheria bacillus, usually in the throat, while in cholera the comma organisms are localised in the intestine. Yet we know the frightful symptoms of lockjaw, the disastrous effects of diphtheria and its prolonged after-symptoms, and in cholera we are acquainted with the quickly fatal course of the disease.

All these severe constitutional symptoms, you will agree, are not to be explained by the local infection and lesion. We may indeed say that these symptoms are characteristic of a poisoning, and that the effects produced on the nervous and other centres that are far removed from the seat of infection and the local nidus of the microbe must be due to an intoxication, a poisoning, of the system by some soluble product of the micro-organism. In such cases the local infection has been followed by a general intoxication or poisoning of the body, which in gravity far outweighs the local symptoms. It has been proved in the case of diphtheria and tetanus that these general symptoms are due to the action of powerful toxins or poisons elaborated by the specific bacteria.

The organisms in other cases may not remain localised, they may spread or creep through a tissue, as in the case of an erysipelas, or they may, as in the case of the

organisms of suppuration spread from a local point through the whole blood, the result being a fatal blood poisoning or septicæmia.

Having considered how the organisms *behave* on entering their host, we have now to consider more closely how they *act*, whether as local or general infective agents, so as to produce the symptoms of disease.

It will help us in this connection to return for a moment to the saprophytic or non-parasitic bacteria—those that live and multiply everywhere except in the animal body. The ordinary saprophytes, if they should gain an entrance into the body, quickly disappear, though some may remain as temporary inhabitants of the digestive tract and derive their sustenance from the food there. Of these certain putrefactive or fermentative bacteria may at times elaborate products which by absorption produce an intoxication of the system. In this way chronic forms of auto-intoxication (self-poisoning) may occur, and may account for occasional attacks of melancholia, when the world seems to us to be out of joint. More acute forms of intoxication may occur through the action of saprophytic bacteria on the food before or after its digestion, resulting in children in milk diarrhœa, or in adults in severe and sometimes fatal cases of meat poisoning, as, for example, occur now and then from tinned foods. You will observe, therefore, that organisms which do not multiply in the body may yet have deleterious effects simply through the products produced by them; they elaborate toxic bodies, through the absorption of which the symptoms are induced. Intoxication, in the ordinary sense of the word, is simply a poisoning of the system due

to the action of the alcohol produced by the yeast plant. Similarly food poisoning is an intoxication of the system due to poisonous bodies or toxins produced by saprophytic bacteria in the food or in the intestine. The causal relation of the microbes to the symptoms is simply in virtue of their chemical products.

In 1856 Panum extracted from putrid meat a non-volatile chemical poison, which produced fatal symptoms in dogs. In addition he also obtained another substance which produced narcosis in animals. These observations were confirmed and similar results obtained by others.

In 1868 Bergmann and Schmiedeberg isolated from decomposing organic matter a substance possessing poisonous properties which they named sepsin.

Selmi obtained from decomposing matter a number of basic bodies giving reactions similar to those of the vegetable alkaloids. For these bodies he suggested the name of ptomaines, derived from the Greek word *πτῶμα*, a corpse. These ptomaines are produced from nitrogenous substances and, as I said, are basic and, like the vegetable alkaloids, such as strychnine, unite with acids to form salts.

In 1876 Nencki made the first analysis of a ptomaine and determined its formula, which was isomeric with that of collidine. Brieger has isolated and analysed a number of such basic bodies from putrid meat, fish, cheese, etc.—*e.g.* neuridine, neurine, cadaverine, putrescene, etc., some toxic, others non-toxic. As stated, certain plants elaborate alkaloidal bodies of a poisonous character, and in the course of the normal metabolism of the human body similar basic bodies are formed, to which Gautier applied the name of leucomaines.

For a considerable time it was believed that the production of ptomaines by bacteria would explain not only the intoxication due to the action of decomposed food, but also the intoxication or poisoning of the system occurring in cases of infectious disease. Brieger devoted much time to investigations on this line, and from typhoid cultures and tetanus cultures obtained minute quantities of basic bodies which he called typhotoxin and tetanin. The results obtained were, however, inconstant, and the possibility was not excluded that the method of isolation might produce such substances. The proteid molecule has a complicated structure, and chemical agents may easily disintegrate it and give rise to a large number of secondary products, due to a rearrangement of the atoms or groups of atoms in the molecule. More important still is the fact that these basic substances cannot fully cover or explain the action of such exquisitely toxic organisms as the diphtheria and tetanus bacilli. The ptomaines have at most a secondary place on the causation of symptoms, though it is possible that their action may make itself felt in cases of food poisoning due to the action of saprophytic bacteria existing outside the body on dead organic matter. And, further, we must note in this connection Van Ermengem's most important discovery of a poison, detected by him during an outbreak of food poisoning produced by ham; he found not only the specific toxin, but also the specific organism to which its formation was due. In specific poisons we probably have the cause of many similar cases of food poisoning where the chief symptoms are not intestinal with diarrhoea, but occur in the central nervous system through absorption

of the toxin. The poison is active in minute doses, is quickly destroyed by temperatures approaching the boiling point, and is not a ptomaine or basic body. I need hardly say this was a most important discovery, and does not favour the hypothesis of the *rôle* played by ptomaines in acute cases of food poisoning, though it is interesting to find that a saprophytic organism may produce a poison in intensity comparable with the toxins of the most virulent infective organisms, such as tetanus and diphtheria.

The above considerations will be sufficient to show that non-parasitic bacteria, when of an injurious character, exercise their action by means of chemical substances that produce an intoxication of the system.

The general study of ptomaines, if it led to disappointment, strengthened at any rate the point of view that the general symptoms of infectious diseases are due to chemical poisons or toxins—or we might go so far as to say that every infection, whether local or general, is accompanied by a greater or less degree of intoxication, and that it is to this that a fatal issue of the infection is due. The production of soluble enzymes by bacteria also aided the theory that toxic disease symptoms might be due to soluble products of their cells.

The study of the specific organisms in the form of pure cultures brought definite results. Thus it was found that the basic bodies from cultures of the tetanus bacillus did not produce effects comparable with those produced by filtered cultures; there was, therefore, a more potent constituent present than the ptomaine.

At the same time it was manifest that the severe symptoms of diphtheria and tetanus are due to some product locally produced by these bacilli, and absorbed into the system. In fact, they have been called toxic diseases because the intoxication is so marked a feature in each case.

It was found that the cultures of these organisms, when entirely freed from the organisms, produced the symptoms of the intoxication observed in the disease; *i.e.* there had been produced in the test tube as in the body, a specific tetanic and a specific diphtheritic poison, capable of acting *sans* bacilli. The tetanus poison, as produced in cultures, *e.g.* in slightly alkaline beef broth, is a most potent toxin, the fatal dose for a man being about $\cdot 23$ of a milligramme, and the average potency obtained from cultures is one hundred times stronger than strychnine. The poison is sensitive to heat, light, and chemical agents—it is an *unstable* body. The diphtheria poison as obtained from broth cultures is also very potent— $0\cdot 4$ milligramme will kill eight guinea pigs or two rabbits. It is also, like the tetanus poison, an unstable body and sensitive to the action of external agents.

The toxins of the tetanus and of the diphtheria bacillus, as obtained from pure cultures, can produce the clinical symptoms of the intoxication due to the action of the bacilli in the body, and in my next lecture I will consider more fully what we know as to the nature of these specific poisons.

What we have already stated shows that in the case of eminently toxic infections, such as diphtheria and tetanus, the toxic symptoms of these diseases are due to specific

poisons elaborated by the specific organisms in each case ; and that, further, the probability is great that in other infectious diseases the severe and fatal symptoms are likewise due to poisons of which our knowledge is as yet incomplete.

The toxins have a great quality in common, viz. the power of producing antitoxins ; *i.e.* when introduced into the body in graduated doses they produce specific substances which neutralise their action. Further, there is an incubation period in their toxic action—hours or days may elapse before the specific toxic symptoms appear.

To be distinguished from these products are poisons contained in the *bodies* of the bacteria—intra-cellular substances. Pfeiffer obtained them in the bodies of the cholera and typhoid organisms, when he carefully killed the bacilli with chloroform vapour. They are not, like the poisons of tetanus and diphtheria, to be found in the filtered, germ-free cultures of the organisms ; they have not been isolated from the body of the cell, and appear to be fixed in it. They are distinguished from the specific toxins we have considered by the fact that the toxic effects of these intra-cellular poisons appear immediately after injection. It may be that they exert a ferment-like action without leaving the cell.

According to Buchner and Hahn, such intra-cellular poisons can be obtained by applying high pressures to the organisms, whereby a cell plasma is obtained. Buchner's experiments on yeast paved the way for this line of research. By using pressures of 400 to 500 atmospheres, he states that he has been able to separate out certain functional from the structural elements of the yeast cell,

and to produce with the cell plasma so obtained an alcoholic fermentation *without* the presence of the living yeast cell. This discovery opens out a wide field of research with regard to the formation of intra-cellular toxins, and possibly also of immunising substances.

We must finally mention a class of substances isolated by chemical means from bacterial cells. I refer to Buchner's proteins. These substances, by a chemical digestion of the cell, were found to be extracts of a stable nature, and not destroyed by boiling, like the specific toxins are. Further, in contradistinction to the specific toxins already mentioned, they appear to be of the same character in many bacteria. Their main action is shown in the production of fever, local inflammation and suppuration, and they exert an attraction upon white blood cells or leucocytes; hence the phenomena they induce in this respect is called leucocytosis.

To this class of bodies also belong tuberculin and mallein, substances obtained from the tubercle and the glanders bacillus respectively.

We see, therefore, that the term 'toxin' has had a varied history. Originally it was applied by Brieger to the poisonous basic bodies or ptomaines. Then it was applied to other kinds of poisons formed by bacteria—the so-called toxalbumins—and the name was transferred to these. But, as we have seen, the specific toxins are probably not albumins, so that, as Ehrlich suggests, the nature of toxalbumins had better be reserved for poisons which are of an undoubted proteid nature, such as abrin, ricin, and snake venom, restricting the term 'toxin' to the specific bacterial poisons, such as those of

diphtheria and tetanus. The work already accomplished proves that specific infective bacteria act in virtue of toxins, and it will be the object of the further lectures to show how this knowledge has been utilised in combating disease. What I have to-day indicated confirms the statement made at the opening of this lecture, that the riddle and its solution lie in the *cell*.

LECTURE II.

The Bacterial Poisons—Ptomaines—Diphtheria and Tetanus Toxins, their Preparation and Nature—Intra-cellular Toxins—Bacterial Proteins—Pyrotoxin—Tuberculin and Mallein.

In the last Lecture the conclusion was reached that specific infective bacteria produce their injurious effects in virtue of toxins. We will now consider more closely these bacterial poisons, how they are obtained, and what we know of their nature and their action in disease. This will lead up to the consideration of the methods for producing immunity to disease, and will help us in our consideration of antitoxins and the lines of treatment that have become associated with this term.

We will begin our special study with a short consideration of those definite chemical entities known as ptomaines or sometimes as cadaveric alkaloids. The ptomaines occur in the course of the putrefaction of organic matter, and share many of the reactions of poisonous alkaloids of a vegetable nature. They are, as you will understand, of importance for forensic medicine where poisoning with plant alkaloids is suspected. The method used for the extraction of vegetable alkaloids from human tissues may also be the means of extracting ptomaines as well, and as some of these ptomaines are toxic, and give alkaloidal reactions, serious errors in diagnosis might readily occur.

A ptomaine might be mistaken for a poisonous plant substance criminally administered. This was brought forcibly to the attention of the Italian Government, and resulted in the appointment of a Commission, in 1879, to consider and to report on the question. The following instances will illustrate what I have just said:—An Italian General, by name Gibbone, died suddenly, and it was suspected by the experts that he had been poisoned by his servant with delphinine or a similar alkaloidal substance. Selmi, however, proved that the basic substance extracted from the dead General, on being treated, gave similar reactions to those of the ptomaines, and that these reactions could not, accordingly, be described as specially indicating the presence of delphinine. Further, that the basic body in question gave reactions with certain reagents that were not given with delphinine, and that, finally, the physiological action of the toxic substance on frogs was different.

Another celebrated case was that of the Widow Sonzogna, of Cremona, who, it was suspected, had been poisoned by the administration of morphine. The redoubtable Selmi, however, proved that the remains contained no traces of morphine, and that the poisonous substance obtained was not morphine, but a ptomaine. In another case that occurred in Verona, Selmi once more was able to show that the strychnine-like body that had been isolated was just as probably a ptomaine. These cases will illustrate the forensic importance that ptomaines possess when the question is one of supposed poisoning with a vegetable alkaloid. But ptomaines are not to be detected exclusively in the human cadaver; they are found

to be products of putrefactive processes generally, and quite a number of such basic bodies have been isolated. We have mentioned already that Panum and others demonstrated their presence in decomposing matter many years ago. The earlier observers obtained them merely in the form of impure extracts, containing, not only other substances, but possibly also mixtures of the bases. Nencki was the first to isolate a chemically pure ptomaine, and to determine its constitution by chemical analysis, and Brieger, by the methods he employed, was able to obtain, in a pure condition, about twenty of these cadaveric alkaloids, and the number has since been added to. The majority of these bodies are what are chemically known as compounds of the amine series. I will not attempt to explain in detail the complicated methods by means of which the ptomaines can be obtained. There are three well-known methods employed for their extraction. We have (1) the Stas-Otto method, in which the basic substance is taken up by means of an organic acid (tartaric acid). We have (2) the Dragendorff method, in which a mineral acid—viz. sulphuric acid—is employed instead of an organic acid. Guareschi and Mosso, however, state that basic bodies are *formed* by the action of sulphuric acid on proteid substances, and they therefore consider the Stas-Otto to be more reliable than the Dragendorff method. We have, lastly, Brieger's method, in which a precipitation of the basic body is obtained by the addition of metallic salts, *e.g.* mercuric chloride. In this way, Brieger isolated many of these basic bodies in the form of double salts. The double salt is subsequently decomposed by sulphuretted hydrogen, which combines with the mercury

to form a sulphide of mercury. The free base is then fixed by means of platinum, or some other body, in the form of a soluble salt. The operations are of a delicate character, and require considerable skill in chemical manipulation, and the use of absolutely pure reagents is essential, for the alcoholic solutions employed for extracting purposes have sometimes been found to contain basic bodies as impurities. We can only place absolute reliance upon any results recorded in this field of investigation when they have been obtained by a thoroughly trained and competent chemist. The ptomaines appear to be formed mainly by the action of bacteria upon proteid matter. Many of the ptomaines are closely related chemically, and it is probable that one may be formed out of another by chemical methods. Thus, by simply removing two atoms of hydrogen and one of oxygen (in other words, water) from choline, another basic body, neurine, is formed. A great deal of research has been devoted to the possible detection of ptomaine in cultures of pathogenic bacteria. Brieger obtained, from cultures of the typhoid bacillus, a crystallisable body, which he named typhotoxin, and which produced toxic symptoms in animals. From cultures of the comma bacillus of Asiatic cholera he obtained quite a number of basic bodies, for two of which he claimed specific characters. The one was non-poisonous, whilst the other had toxic properties causing diarrhoea and a depression of the body temperature. From tetanus cultures Brieger likewise isolated five basic bodies, amongst which was tetanin, a tetanic alkaloid. The ptomaines are extraneous substances formed by bacteria out of the soil in which they grow,

and the quality of the soil has therefore an influence upon the amount of such bodies that may be produced : thus a decoction of meat appears to give the best results. They are bodies which probably many forms of bacteria are capable of producing, and are not therefore to be regarded as of a specific nature. The production of a specific ptomaine in connection with an infectious disease has never been proved. We shall not, therefore, have occasion to make further reference to the ptomaines in these lectures.

We pass to the consideration of those poisonous bodies of a specific nature that are associated with specific infectious diseases, and we will confine our attention to those of which our knowledge is of a definite nature. I refer more especially to the toxins of those eminently toxic diseases, diphtheria and tetanus. Each of these affections is due to the action of a specific organism. In the case of diphtheria the agent is the Klebs-Löffler bacillus ; in the case of lockjaw it is the tetanus bacillus, first detected by Nicolaier, and subsequently isolated by Kitasato. It may be of interest to describe the method by which cultures of these organisms are obtained. The diphtheria bacillus is obtained directly from the throat of a person suffering from the disease by means of a swab applied to the back of the throat, the result being that a certain number of the bacilli adhere to the swab. The swab is then rubbed over the surface of a tube containing solidified blood serum, and in this way the bacilli are transferred to a suitable culture soil. The tube is then placed in an incubator overnight at blood heat, because such pathogenic microbes grow best at the temperature of the body.

On the following morning there is a visible growth of bacteria, amongst which are to be found colonies of the diphtheria bacillus. With the aid of a platinum wire needle one of these colonies is removed and seeded in a fresh sterile soil, and an absolutely pure strain of the diphtheria bacillus is thus started.

For the purpose of obtaining the *soluble* toxic products of the bacillus it is necessary to cultivate the organism in a *fluid* soil. Beef broth is the most convenient medium for this purpose, and in order to obtain good toxins the broth solution should be free from sugary matter. The presence of sugar frequently results in the production of acids that hinder the growth of the bacilli and lessen their activity. Different strains of diphtheria bacilli vary in their toxin-producing properties—some yield toxins of high potency, some furnish toxins of a medium strength, whilst in not a few cases the toxins are of a comparatively feeble character.

After a week or longer incubation of the flask at blood heat, a toxin-containing fluid is obtained. We note a deposit of bacilli in the flask and a film of young bacilli on the surface of the fluid. If we desire to obtain the soluble toxins free from the bodies of the bacilli we proceed as follows:—The entire culture is filtered through a Berkefeld filter. The Berkefeld filter retains in its pores the suspended matter, including the micro-organisms, and a clear, sterile fluid passes through into the receiver; this fluid contains the soluble poison. It is placed in a sterile vessel, and as the toxin is an unstable body, readily affected by light, heat, and air, it is preserved in a cool and dark place and the air excluded by means of a thick layer of

an antiseptic substance called toluol. The fatal dose of an average diphtheria toxin is about one to two drops for an animal of the size of a guinea pig, but the more virulent toxins obtained are fatal in much smaller doses.

The tetanus bacillus is obtained from the body of an infected person or animal. The conditions for its growth are different from those of the diphtheria bacillus, for while the diphtheria bacillus grows freely in contact with air, the tetanus bacillus refuses to grow under such circumstances; it requires for its development the exclusion of air or free oxygen. To those organisms that grow only in the presence of air the term *aërobic* is applied; to those that grow only when air is excluded the term *anaërobic* is applied, *e.g.* the tetanus bacillus. We have also an intermediate group capable of development under either conditions, with or without air; these are known as facultative organisms. How do we obtain the conditions for the anaërobic growth of organisms? This may be accomplished in several ways:—

1. By mechanical exclusion of the air, the cultivation being made in the depth of the culture medium.

2. By absorption of the oxygen from the air present in the flask, by the use of a mixture of pyrogallie acid and caustic potash.

3. By driving out the air and substituting an indifferent gas, such as hydrogen. In such a flask the tetanus bacilli are sown, the medium being peptone broth. After three or four weeks the fluid is passed through a Berkefeld filter, and the soluble tetanus toxin obtained, of which $\frac{1}{4000}$ th part of a cubic centimetre is a fatal dose. It will be one of the main objects of my next lecture to explain

how these toxins are utilised in the preparation of antitoxins. The toxins can be precipitated out of their solutions by the addition of alcohol, or by saturating the fluid with certain salts, such as ammonium sulphate. An amorphous powder is obtained, consisting of toxin and precipitated matter. The toxin is simply entangled and carried down with the precipitate of proteid substances resulting from the addition of the alcohol or ammonium sulphate to the fluid in which it is dissolved. These toxins have not yet been obtained as definite chemical entities, as is the case with the ptomaines, their exact chemical constitution is still unknown, and we have to rely upon physiological tests for their detection. The physiological test has proved that cultures of the diphtheria bacillus contain a soluble specific poison, which is capable of reproducing the toxic effects of this disease. The poison can be precipitated out of its solutions, as I have said, and it does not dialyse through parchment; it is not, therefore, of the nature of a peptone. The poison is most sensitive to heat, and is quickly destroyed by temperatures approaching the boiling point. The poison was originally classified amongst the toxalbumins on account of the proteid reactions which it gives, but it is probable that these reactions are due to impurities of a proteid nature adhering to the poison after its precipitation. Thus Brieger and Boer succeeded in precipitating the toxin of diphtheria from cultures of the organisms in the form of a zinc compound; it was then found that the poison did not give the typical proteid reactions. The physiological test has similarly demonstrated that the tetanus bacillus produces a specific, soluble tetanic poison. Brieger and Cohn afterwards succeeded in pre-

precipitating the toxin from its solutions by means of chloride of zinc and in purifying it from proteids and peptones. This purified tetanic poison, on being tested, did *not* give the typical proteid reactions. The conclusion we must come to, in the light of these investigations, is that neither the diphtheritic nor the tetanic poison is a genuine proteid or toxalbumin. The tetanus poison, when obtained in a purified form by means of chloride of zinc, has most remarkable toxic properties—a millionth part of a gramme, or an imponderable quantity, being sufficient to kill a mouse.

Roux and Yersin supposed the diphtheria poison to be of the nature of a ferment or enzyme, and it certainly shares with the enzymes their sensitiveness to external influences, such as heat, light, and chemical agents, and the faculty of exercising its action in minute amounts. It is of importance to note that Guinochet, Uschinsky, and others have proved that the toxic organisms still produce their toxins when cultivated on a soil entirely free from proteid matter. We cannot, therefore, regard these specific toxins as genuine proteid or albuminous bodies, nor can we regard them as products formed out of the proteid matter on which the organisms are grown, inasmuch as they are still formed on a non-proteid soil; they may rather be considered integral constituents of the body of the bacterial cell, or immediate derivatives of these constituents. Another theory propounded is that the specific poison, as in diphtheria and tetanus, is formed out of the proteids of the tissues of the body by the action of a ferment or enzyme secreted by the micro-organisms. The theory most generally favoured, however,

is that the specific toxins are synthetic products formed in and secreted by the bacterial cell.

The specific toxins with which we are acquainted have two common properties—(1) the capacity to induce the formation of specific anti-toxins that neutralise their action, and (2) the incubation period that ensues before their toxic action manifests itself in the system.

There are other poisonous substances present in the bacterial cell of an unstable nature, and readily affected by heat. These intra-cellular or primary poisons, as they are called, are undoubtedly formed within the cell, and our knowledge of them is mainly due to the investigations of Pfeiffer. These poisons are obtained by carefully killing fresh cultures of the cholera or typhoid organism by means of chloroform vapour; they are not to be detected in filtered cultures of the organisms. Upon heating to 60° C. the activity of such intra-cellular poisons is diminished, but is not entirely abolished. There remain, according to Pfeiffer, secondary toxins of a more stable character, but ten to twenty times less toxic than the primary poisons.

These bodies have an immediate toxic effect, without the incubation period observed in connection with the toxins of diphtheria and tetanus, and the fatal effect is produced by a dose of 8 to 10 milligrams. Pfeiffer believes that in *Cholera Asiatica* the toxic phenomena are due to the absorption of the intra-cellular poisons of the comma bacillus.

There is no doubt that the intra-cellular products of many micro-organisms have a distinct and marked physiological action. We have already referred to Buchner's

experiment, and the alcoholic ferment separated by him from yeast-cells by means of high pressures exerted directly on these organisms. Similar experiments are being carried out upon disease germs, and the results will be of great interest and value in elucidating the nature and origin of the bacterial toxins.

Our survey would not be complete without reference to another class of substances contained within the bacterial cell, which are of a more resistant character than those we have just been dealing with. I refer to the Proteins, which are obtained in the following manner. The micro-organisms—*e.g.* *Bacillus pyocyaneus*—are rubbed up with water, and a half per cent. solution of caustic potash is added to the emulsion. A mucilaginous mass is obtained, which dissolves on heating, and which is clarified by filtration. If this solution be rendered faintly acid, a precipitate of the dissolved protein occurs. This precipitate is washed and once more dissolved by the addition of a weak alkali, so as to form about a 10 per cent. solution of the protein. These proteins are termed alkali-proteins, to distinguish them from proteins not precipitated in acid solutions. The latter, obtained from many kinds of bacteria, are prepared by first drying the micro-organisms quickly, then the dried mass is rubbed up with water and heated for an hour and a half at or over boiling point, and the mixture is finally filtered. Their action is not of a specific nature, the main symptoms they produce being fever, local inflammation and suppuration; and chemically they approach in nature the plant caseins. Old broth cultures of bacteria contain them; here they have oozed out from the disintegrating bodies of dead bacilli. The

formation of pus in the body may be coincident with such a disintegration of the pus-organisms in the tissues. This is, however, a property shared by many different races of bacteria, and suppurative processes are not so closely bound up with the action of a given specific organism as is the case with the toxic diseases. Tuberculin and mallein also are closely related to these bacterial extracts. These substances are glycerin extracts prepared from the bodies of the tubercle bacillus and the glanders bacillus respectively, and, like the proteins, they are resistant to the action of heat. The ordinary tuberculin is prepared by cultivating tubercle bacilli in a broth containing glycerin for six to eight weeks. The bacilli are digested by boiling the culture, which is then filtered to remove the disintegrated bacilli, and the fluid is concentrated to one-tenth of its volume. The mallein is prepared in a similar fashion from glanders bacilli. A minute dose of tuberculin has no apparent effect on a healthy animal, but if the animal is suffering from tuberculosis a transient, though marked, fever occurs, accompanied by a local reaction at the tuberculous focus, characterised by redness and swelling. The tuberculin is now mainly employed for the purpose of diagnosing tuberculosis in cattle. It is undoubtedly of great value in picking out affected animals amongst a herd of cattle, or in a dairy farm, when they are still in the early stages of the disease. In other words, animals which through their milk or secretions are in a position to communicate the infective agents, not only to other cattle, but also to man, can thus be sorted out.

Mallein performs an equally useful service in aiding the early diagnosis of glanders in horses. It produces a

transient fever, and a local swelling in an animal suffering from this disease. The new tuberculin of Koch is prepared in a different way. The old tuberculin represented the constituents of the tubercle bacilli that are soluble in glycerin. The new tuberculin represents certain constituents of the tubercle bacillus, which are obtained by pounding up and extracting virulent bacilli. It does not give the fever reaction characteristic of the old tuberculin. Its therapeutic use has been disappointing, but it may possibly be found of use in immunising animals, with the view of obtaining some antitoxic principle.

Fever is a general symptom of infectious diseases, and we will lastly mention a substance obtained by Centanni from the bodies of a number of bacteria. The main symptom this substance induces is fever, and hence Centanni christened it *pyrotoxin*. These results require further confirmation; but they are of an interesting nature. On surveying the ground traversed by us in the course of this lecture we find that the poisonous substances produced by the action of bacteria are of various characters and properties; some are formed out of the medium in which the organism is growing, others appear to be secretions of the cells which can leave the cell and pass into the outside medium, whilst others exert their action as intra-cellular poisons fixed within the cell. We have, therefore, to distinguish :—

1. The ptomaines or basic bodies.
2. The specific toxins, such as those of diphtheria and tetanus, which are very sensitive to heat and to external conditions, and which are to be obtained in cultures of the specific organisms.

3. The intra-cellular poisons, obtained by Pfeiffer from the cholera and typhoid organisms. These bodies are also sensitive to external agents and easily modified by heat into less active or secondary poisons.

4. Certain portions of the cell framework or the substance of bacteria—the bacterial proteins—which produce symptoms common to the action of many organisms, *e.g.* inflammation and fever. Tuberculin, mallein, and pyrotoxin are closely allied to these bodies. In contradistinction to the specific toxins they are resistant to the action of external agents and heat.

We are brought to the conclusion that an organism in its deleterious action on the tissues of the body acts in virtue of some product of a poisonous nature, and that the distinction between infection and intoxication is a more or less arbitrary one. Intoxications of the system can occur *without* infection, and infections rapidly assume the features of an intoxication. You will therefore appreciate the importance the investigation of such poisonous bodies has for us in the endeavour to explain and to combat the symptoms of disease. This must be my excuse for calling attention so particularly to the bacterial toxins. I have directed your attention to the shady side of the picture in describing the attacks to which the human frame is liable from without. I have dealt with the living agents that produce disease, and explained the nature of their weapons.

The tissues and their cells are not, however, without means of defence whereby these attacks are repelled or their effects modified.

The body naturally possesses, or may artificially acquire, valuable weapons of defence.

To explain what these are and how they act will be the first aim of my next lecture, and this will lead us naturally to the consideration of antitoxins and their *rôle* in the treatment of disease.

LECTURE III.

The Defences of the Body against Bacterial Invasion—Immunity—
Germicidal Action of Blood Serum—Phagocytosis—Protective
Inoculation—Acquired Immunity—Antitoxin Production.

Having considered the attacks to which the human body is liable from without by living cellular agents, and the nature of the weapons they employ, we indicated that no living cell is without some means of defence, and that the cells of the body possess naturally, or may acquire by painful experience, means of protection against these insidious foes. To explain these means of defence and their mode of action will be one of the main objects of this lecture. The air we breathe, the water we drink, the food we eat, and, indeed, most objects with which we come in contact, contain microscopic forms of life, amongst which are constantly to be found various forms of bacteria. Through the channels I have just mentioned these bacteria may be transferred to and may become messmates of the human body. The entire surface of the body normally harbours not only harmless forms of bacteria, but also types which are recognised to be specific causes of inflammation and suppuration. Each one of us unwittingly possesses a botanical garden filled with a diverse and a varied bacterial flora. The attempt was, I believe, once made by an ardent student in this field to

enumerate the various bacterial forms to be met with on the skin, but the task proved too much for him before the hundredth species had been reached. This will lend force to the remark once made to me by a distinguished man that he never came away from a bacteriological lecture without feeling like a mouldy Stilton cheese. The intact skin does not, however, permit these organisms to penetrate much below the surface, and it forms a valuable protective covering to the tissues. Numerous investigations have shown that the tissues of the body are normally practically free from bacteria, and that this is so is largely due to the intactness of the epidermis. If, however, some lesion of the skin should occur—a solution in its continuity by means of a cut, a crack, or a wound—an entrance channel is immediately afforded to the organisms vegetating on the surface of the skin. A microscopic lesion is sufficient to allow of the penetration of such minute forms, and in this way an erysipelas may be set up, an abscess, or a boil, or a more general infection such as anthrax, plague, or blood-poisoning may take place. It is an undoubted fact that the plague bacillus is frequently introduced into the system of Asiatics by wounds in the skin. The lowest class of Bombay natives lives in crowded dwellings under the most filthy conditions, and are afflicted with certain troubles to which scratching gives a momentary relief. The plague is eminently a dirt and house disease, and you can readily understand how the natives are being constantly inoculated with the specific bacillus in the manner I have suggested. Beneath the skin we have a copious system of blood and lymph vessels ready to absorb any infection and to generalise it; the

intact skin is, therefore, a valuable barrier against infective agents, and its action is mainly of a mechanical nature—it is naturally impervious. Stinging insects may, however, overcome this barrier and transfer organisms to the subcutaneous tissues, whence they are absorbed into the system. Thus it is known that mosquitoes may transfer the specific malaria parasite from one person to another. The skin is, however, under everyday circumstances a valuable means of protection; we may term it our first line of defence.

Further, in the mouth, the upper portions of the respiratory tract, and in the whole of the digestive system, we find a multitude of bacteria, some of which possess infective properties. For example, the diphtheria bacillus and the organisms that produce certain forms of pneumonia are to be met with at times in the throats of perfectly healthy individuals. The throat and the digestive tract are, however, furnished with a delicate external lining membrane, which in an intact condition does not allow organisms to penetrate into the tissues beneath. If any break in this lining membrane should occur, the organisms adhering to it may find their way into the tissues and, if of a sufficiently virulent character, produce disease. The intact surfaces of the throat and digestive organs act, therefore, as a valuable means of defence; they constitute an internal skin. The nose is one of the most efficient germ filters Nature has furnished us with; bacteria inhaled with the dust of the air rapidly disappear in the nose and do not pass on into the lungs. To breathe through the nose is not only important physiologically as a means of warming the inspired air; it is also important

as a means of ridding the air we breathe of many living germs. Some of the secretions of the digestive tract exercise an antiseptic action. Thus the gastric juice has an acid reaction, due to the presence of hydrochloric acid. Most of the organisms that produce disease are sensitive to the action of acids, and we have, therefore, when cultivating them in the laboratory to ensure that the soil in which they are grown has a neutral or slightly alkaline reaction. The cholera organism, for example, absolutely refuses to grow in the presence of a free acid. If, therefore, infective bacteria are exposed to the action of the acid gastric juice for any length of time, their vitality is either weakened or entirely destroyed. The lower portions of the digestive tract frequently play an important part in ridding the system of injurious material. The diarrhœas that occur from time to time, as well as the attacks of vomiting, are frequently Nature's attempts to get rid of some noxious agent. The emetic and the purge have, therefore, their *raison d'être* in preventive medicine; and one can appreciate the method of treatment adopted by a certain naval surgeon: if the patient complained of pain above the middle line of the body he promptly administered an emetic, if the symptoms occurred below the critical zone a good dose of jalap was given.

We have up to the present considered mechanical defensive agents such as the skin, or purely chemical agents such as the acid of the gastric juice. We will now pass to the consideration of the means of defence that are called into play when an infective agent actually gains admittance into the tissues of the body. It will be advisable in the first instance to refer to words which have

become current coin in this connection, and to define exactly the meaning that is to be attached to them. I refer especially to the terms 'immune' and 'immunity.' *Immune* (Latin *immunis*) signifies a freedom from certain burdens, punishments, &c., and likewise a freedom from infection by morbid agents, such a condition being known as *immunity*. In the middle ages the term meant, amongst other things, freedom from certain public obligations which it fell to the lot of the ordinary member of the community to fulfil—for example, the exemptions accorded to Royal demesnes and Church possessions. This immunity extended also to certain individuals as regards taxation or punishments. In a much later and a purely physiological sense immunity means the exemption of the body from the action of certain morbid influences, especially those of an infectious nature; in other words, the absolute or relative insusceptibility of the living organism to the attacks of a given infective or microbial disease. This immunity is most marked in connection with infectious diseases. It is a familiar observation that not every individual exposed to infection with scarlet fever or diphtheria acquires the disease; some remain temporarily or permanently unaffected by these diseases though exposed to the contagion, others readily become affected with them. We notice this amongst children—the members of one family appear to catch every ailment that prevails, whilst in other cases a complete exemption occurs. Further, we find that animals are insusceptible to many diseases to which the human race is liable—for example, such acute diseases as scarlet fever, typhoid fever, and measles are not known amongst the lower animals, and,

conversely, many diseases are peculiar to animals, and do not affect man—*e.g.* blackleg or quarter evil in cattle. Differences in disposition likewise occur, based on racial peculiarities; thus birds do not naturally become affected with anthrax, a disease to which cattle are frequently liable. The white man falls a victim to yellow fever, whilst the black man largely escapes. In all the cases I have cited *Nature* has herself provided an exemption, an immunity, to the given disease. We speak, therefore, of the individual who, though fully exposed to the contagion, escapes cholera, as being naturally *immune*, and, generally speaking, the individual or animal that proves exempt from a given disease, as immune. The condition of the individual escaping cholera, and the condition exempting an animal or human being from a certain disease, is described as natural immunity, and the individual or the class to which it may belong is said to possess a *natural* immunity to the disease in question.

Besides this natural immunity another form of immunity also occurs. A number of infectious diseases attack the system only once. Thus an attack of scarlet fever, measles, or smallpox occurs generally only once in the lifetime of an individual; the recovery from the disease has resulted in a protection against subsequent attacks of the same ailment. In other words, a condition of immunity has been left behind which has been *acquired* through the effects of the disease on the system. In such cases we speak of a natural *acquired immunity*, in contradistinction to the *natural immunity* which pre-exists towards various diseases in man and animals, which is not acquired, but which is inherent in the system.

When the attempt is made by means of special vaccines to produce immunity to a given disease in an animal otherwise susceptible to that disease, and the result is successful, the immunity that is thus brought about is termed an artificial acquired immunity, inasmuch as it was intentionally produced. A classical example of this is to be found in the methods of vaccination against smallpox. We have, therefore, to bear in mind the significance of these various terms. An insusceptibility to the attacks of a given disease is termed immunity, and the individual or species concerned is said to be immune. This insusceptibility may be already ingrained in a class of animals or an individual, and the condition is then described as one of natural immunity. The insusceptibility may be acquired after an attack of a given disease, and the condition is then termed an acquired immunity, or, finally, it may be induced by the introduction of a vaccine into the system, as in the case of vaccination against smallpox, when the condition is known as an artificial immunity. Having explained the significance of terms which we shall make frequent use of in the course of this lecture, we may proceed to consider the principles that underlie and give to these terms their special significance. An immunity to noxious agents is not confined to man or to the higher animals. Many unicellular plants and animals are exposed to attacks from without which may terminate in some injurious effect or may not. We are not in a position to state much of a positive nature with reference to the relative resistance or immunity of the individual species of lower organisms to agencies of a harmful character; but that they are capable of producing injurious effects upon

their foes is beyond doubt. The struggle for existence that is constantly going on in nature necessitates the development of means of defence ; this may assume forms so far removed as the colouring imitative of surrounding objects found amongst insects, and the more subtle processes developed in the cell. The more active these cellular processes are the greater are the chances of survival.

Unicellular organisms vary in their behaviour towards poisons. The bacterial cells are very sensitive to the action of various mineral poisons, but considerable differences occur in the degree of sensibility exhibited ; thus a dose sufficient to kill one species may be inadequate for another. In estimating the value of a disinfectant, its exact capacity to do the work it is intended to accomplish must therefore be known. And we have a very delicate control for that purpose, which is carried out by testing the action of the disinfectant upon pure strains of various bacteria, and a new disinfectant nowadays is thus tested in the bacteriological laboratory. These more accurate controls have shown that many so-called disinfectants are worthless for the specific purposes for which they have been used, and the knowledge thus gained has proved naturally most valuable from a practical point of view. It has been found that bacteria may accustom themselves to an otherwise fatal dose of a poison ; thus organisms submitted to the progressive action of an antiseptic may ultimately acquire the power of living in a solution in which the untrained organism would quickly die ; a toleration and immunity have been established towards the poisonous substance. Effront, in this connection, has proved

that beer yeasts can be accustomed to grow in amounts of hydrofluoric acid fatal to the unacclimatised forms: that is, they acquire an immunity by the treatment. This immunity was found to be hereditary, the resistant cells transmitting this property to their progeny; and, further, such cells exhibited more active fermentative properties than the ordinary yeast cells. We find, therefore, amongst simple cells striking examples of an acquired immunity to noxious agents, and illustrations of the power a living cell has within certain limits to adapt itself successfully to an unfavourable environment. In this connection, and dealing with the observed adaptability of a simple cell, we must consider the acquired immunity as due to certain properties developed within the cell itself: in other words, as an intra-cellular phenomenon, and one to be observed even amongst the lowest forms of life. We referred to natural immunity—that condition of the body which renders man immune to certain diseases that affect the lower animals, and which also protects the lower animals against attacks of disease to which higher beings are liable. Much research has been devoted to the elucidation of the causes underlying natural immunity. We noted that in a disease process we had to consider two factors: (1) the infection, and (2) the intoxication, *i.e.* the action both of the living agent and of its products, and it is to be noted that in these respects the resistance of the body varies. The tissues may be immune to the attack of an organism and also to its toxins, but, on the other hand, the tissues, while immune to an infecting agent, may be susceptible to the toxins produced by it. Thus we find that rats and mice are highly insusceptible, not

only to infection with the diphtheria bacillus, but also to any intoxication with its products: in other words, these animals possess a natural immunity. On the other hand, the tissues, while immune to the living agent, may be very susceptible to its poisons, *e.g.* the *Bacillus prodigiosus* is harmless to man, but its toxins produce, when introduced into the tissues, distinct poisonous effects. We find in nature that the natural resistance or immunity to the living agents of disease is a much more common thing than the resistance or immunity to the toxins they produce. Indeed, if we could rob the micro-organisms of their toxin-producing properties the tissues would, in most instances, easily dispose of them, as is the case with other kinds of foreign and adventitious matter. The simplest explanation of a natural immunity to disease agents is the supposition that it depends upon some inherent quality of the fluids of the body, viz. the blood and the lymph. In other words, the blood or lymphatic fluids form an unsuitable nidus for the growth and development of the parasites in question. This theory is one that held favour for a number of years, and has been vigorously defended. Nuttall, in a series of experiments, showed that the blood serum of many animals has an injurious effect on such an eminently pathogenic organism as the anthrax bacillus. The blood in contact with these bacilli kills them in virtue of some antiseptic property that it possesses. When, however, the blood was heated to 55°C . this antiseptic action ceased, due to the destruction of the antiseptic substance. Many researches have been carried out on similar lines since Nuttall first made this discovery with a view of demonstrating antiseptic properties of the

blood. Thus it was found that the blood of the white rat exerted a most destructive action on the anthrax bacillus, and this was held to explain the natural immunity this animal possesses to the disease. Buchner went still further in the generalisation of this theory, and sought to demonstrate that there were actual bactericidal substances in the blood of unknown chemical constitution, which he termed 'Alexins.' Their full action is only exerted in the presence of certain salts: if these are removed from the blood the action ceases; if restored, the action reappears. If blood is heated to 55° to 60° C. their action is destroyed, and they can be precipitated from the blood on the addition of alcohol. This antiseptic action Buchner found could be demonstrated in samples of blood outside the body. This theory would have given an admirable and simple explanation of natural immunity. Unfortunately, on attempting to generalise the observations originally made, contradictory results appeared. The rabbit's blood exerts an antiseptic action on anthrax bacilli, but, despite this, the animal is easily infected with the organism; dog's blood has no injurious effect on the anthrax bacillus, yet this animal is immune to the bacillus and the disease. One cannot therefore, consider a purely antiseptic property residing in the blood as affording a general explanation of the natural resistance so widely observed to certain diseases. The wonderful thing is not the few diseases we acquire, but the many we escape. This simple and purely humoral theory thus received a heavy blow. Buchner and many others continued their experiments. They found that fluids in the body which were rich in cellular elements, termed leucocytes, were more deadly for a given organism than

the cell-free fluids in the body of the same animal; the killing, the antiseptic, action of a body fluid was immediately increased on the addition of the cellular elements, the leucocytes. Further, body fluids which contained a large number of these corpuscular elements possessed strong antiseptic properties. In fact a certain relation existed between the antiseptic action of the fluid and the number of corpuscular elements or leucocytes present. Buchner originally believed antiseptic power was an essential property of the fluid elements of the blood, but in view of these observations he modified his theory in the following manner:—The alexins or antiseptic bodies do certainly exist in the fluid blood and act there, but they are not preformed in the blood, they are secretive products of the living cells or leucocytes that exist in the blood. These free cells, on the occurrence of a local infection, hurry to the point of invasion, and secrete antiseptic substances which, passing into solution in the fluid blood, destroy the micro-organisms—they separate out soluble substances which exert their action in the blood plasma. Buchner would therefore negative the theory that the cellular elements existing in the blood have any direct part in the destruction of foreign elements such as bacteria. If bacteria are introduced into an animal naturally immune to these bacteria, it is generally observed that a certain number of them die, and are taken up by some of the amœboid wandering cells of the body. The cells exercising this action on the invading bacteria are termed phagocytes; the process is phagocytosis. There are both wandering phagocytes, which are capable of moving about to different parts of the body,

and also anchored phagocytes, as, *e.g.*, in the spleen tissue and bone marrow. In a naturally immune animal, when the microbes come in the neighbourhood of such cells, they are taken up, ingested, and disappear. If at the given point no phagocytes are present, or if they are too few in number, others wander to the point of invasion, and help in ridding the body of the invading organisms. This is rendered possible by the action of the bacteria on the tissue, which results in certain inflammatory changes. This acts as a danger signal, and is the means of attracting an army of phagocytes to the threatened spot. Phagocytosis is a widely observed phenomenon, to which Metchnikoff has devoted a lifelong study. In this way the anthrax bacilli are greedily devoured by wandering cells or phagocytes. The process, according to Metchnikoff, is an intra-cellular one. The phagocytes possess ready-formed deleterious substances, or they may form them when an emergency arises, and if they are broken up or die, these substances may be liberated, and probably represent a great part of the antiseptic substances present in the blood—the alexins of Buchner. Before the phagocytes are in a position to assert their action certain preparatory phenomena occur, which are rendered possible through the great sensitiveness of phagocytes to external influences. The anchored phagocytes stretch out arms of protoplasm, which surround and absorb bacteria. The motile phagocytes must, of course, first come to close quarters with the enemy. These cells have the property of feeling and reacting sympathetically or otherwise to chemical constituents in their environment; they are attracted by some substances—positive chemotaxis; they

are driven away by others—negative chemotaxis (see also p. 249). The phagocytes of a naturally immune animal exhibit the positive property, the substances formed in inflammatory changes and produced by bacteria having the effect of attracting them, and they thus come to close quarters with and absorb the organisms into their cell substance and render them innocuous. If their vitality be lowered the natural protection may cease: *e.g.* exposure to cold favours infection with anthrax. Metchnikoff ascribes an important rôle to these fixed and wandering cells in *natural* immunity (we are here, of course, dealing strictly with an immunity to the living germ or infective agent). You will see, therefore, that with regard to natural immunity, three theories have been advanced by way of explanation—(1) That protection is due to antiseptic substances present in body fluids, *e.g.* the alexins. This is a purely humoral theory. (2) Directly opposed to this view is a purely *cellular* theory—viz. that the resistance to attack is due to certain fixed or wandering cells of the body, which at the point of invasion ingest and destroy the living infective agents. (3) The third theory seeks to throw a bridge between these opposing views. This suggests that the cells do not act directly, but indirectly by means of substances they secrete. This is a combination of the humoral and cellular theories. The substances secreted by the cells act in the body fluids and weaken or destroy the organisms, and the phagocytes then take them up just as they do any dead matter; they are simply scavengers.

A few words must now be devoted to natural *acquired* immunity. Protective methods of inoculation originated

from the observation of what occurred during outbreaks of epidemic disease—viz. the fact that individuals after passing through an attack of a given disease, were for a time, or permanently, protected against a recurrence of the same disease. This immunity, it was found, also occurred, even when the attack of the disease was of a mild character; thus a slight attack of smallpox protects against fully virulent smallpox virus. This suggested the idea, followed out in the earliest times, of utilising mild epidemics of a disease as a means of producing artificial attacks in healthy persons, and in this way obtaining some protection against later, and possibly more serious, outbreaks. In India this form of protection against smallpox was long used by the natives. In human beings, after attacks of cholera, typhoid fever, and diphtheria, immunising substances may be found in the blood, at least for a time. In the cholera blood an anti-infectious substance may be found, and similarly in cases of typhoid fever the blood may acquire immunising properties, and, after an attack of diphtheria, the blood may contain anti-toxic substances. Indeed, in many instances, the crisis in a disease appears to be coincident with a self-immunisation of the body to the toxic products of the infective agents.

The blood of an animal rendered immune to the cholera or typhoid organisms, acquires remarkable properties of a specific character, whereby the fluid portion of the blood quickly kills the typhoid or cholera organisms; they degenerate under its influence, break up and disappear, much in the same way as a lump of sugar dissolves up in water. It is, however, only cholera blood that acts in

this way on cholera bacilli, and only typhoid blood that acts on typhoid bacilli. The action is, therefore, specific, and is known as 'Pfeiffer's Phenomenon.' Further, the blood from a diphtheria patient can frequently neutralise and render inert the toxins of the diphtheria bacilli in virtue of antitoxic substances present in it. The blood has, therefore, a varied and marked action on bacteria and their products.

We must now proceed to discuss shortly the various means that have been adopted to imitate, if possible, Nature's methods of defence; in other words, the attempts made to artificially produce immunity to a disease. Following the Jennerian tradition, in connection with smallpox, the attempt was made to protect an animal against an attack of a virulent disease by inducing in it a mild attack of the same disease. The old observation that a mild attack of smallpox was capable of affording protection, led in the early days to variolation, and subsequently to vaccination. Many investigators followed the new line of treatment thus opened up. The endeavours were aided by the discovery of the specific agents in many infectious diseases, and the possibility that was thereby afforded of direct experiment in the laboratory on the living virus.

It was attempted to attenuate the natural virulence of pathogenic bacteria by means of various agents, such as heat and chemical substances, and in this way vaccines of graded degrees of virulence were produced, the vaccines consisting of the attenuated or weakened living virus. Pasteur, by growing anthrax bacilli at high temperatures, obtained an attenuated form of the bacillus, which he used for the inoculation of cattle which are subject to this

disease. In this way a mild disturbance of the system took place which strengthened the animal against the attack of the fully virulent virus, and in chicken cholera the same line of procedure was adopted. It was found, further, that not only the attenuated living virus, but also its sterilised or filtered cultures could afford a certain degree of protection, *e.g.* with *Bacillus pyocyaneus* and blackleg or quarter evil. We see already, in these earlier observations, a recognition of the part played by toxins, not only in the production, but also in the prevention, of an infectious disease. Following the idea of utilising attenuated bacterial cultures as a means of protection against disease, Pasteur extended his observations to hydrophobia, and was enabled by the use of certain means to attenuate the unknown virus of this dreadful disease, and to introduce a system of preventive treatment for rabies which has proved markedly successful.

If we consider the general features of an acquired immunity that has been produced by artificial means, it appears to result from some irritating stimulus given to the living cells, to which they react with the production of substances that have the power of rendering inert or innocuous the living agents of the disease. Pfeiffer supposes that the antiseptic action of the blood of immunised animals is not an inherent property, but that the blood contains an anti-infective body in an inactive condition, and that this inactive body is first changed into an active antiseptic substance within the system of the inoculated animal by means of some ferment which its cells secrete. In protected animals it is found that phagocytosis is more marked than in unprotected animals. Metchnikoff

supposes that in artificial immunity some substance is probably produced that protects the phagocytes and allows them to carry on their work unhindered.

Behring found that animals could be protected against the diphtheria bacillus without being rendered immune to the toxin ; and similarly it was found that animals could be protected against the typhoid and cholera organisms, but that they still retained susceptibility to the toxins of these organisms. We therefore see that an artificially-acquired immunity to living infective agents can exist, despite the susceptibility to the action of their toxins, just as is the case in natural immunity. Toxin immunity is rarer than bacterial immunity, though it does exist and can be acquired. How is it that an artificial immunity to toxins is to be obtained? We have familiar examples of an acquired toleration to poisons. Thus, in certain mountainous districts, the inhabitants have acquired a toleration for doses of arsenic that are usually fatal to the ordinary individual, whilst we know what large quantities of opium can ultimately be tolerated by an opium eater. We saw that after an attack of diphtheria the blood contains substances which do not act on the micro-organisms, but on their metabolic products, the toxins—hence the name of antitoxins given to such protective substances—and that the recovery from the grave general symptoms of the disease was most probably due to the formation of these substances, which neutralised the poison and prevented a fatal result.¹ If these antitoxins

¹ Later work has not substantiated this hypothesis. Antitoxin is not found in the blood of diphtheria patients until the second week of convalescence, and hence can have little to do with the recovery from the disease.—ED.

were produced in sufficient quantity the individual recovered, if not, the individual succumbed to the disease.

The idea, therefore, occurred to Behring that if we could ensure the presence of the right amount of antitoxin in cases of tetanus and diphtheria, the specific poisons of these diseases would be neutralised, and recovery would take place. Could we not artificially introduce into the system of a patient the antitoxin that his system might not prove capable of producing in the right amount? Behring found that by introducing in carefully graduated doses the toxins of diphtheria and tetanus into animals he was able to induce in the animal a reaction which resulted in the formation by the body of antitoxic substances, which rendered it immune to otherwise fatal doses of the toxin—the bane produced its own antidote. Behring further found that this immunity could be transferred to other animals; thus, if he injected a small quantity of the blood of the immunised animal into an unprotected animal it acquired the same resistance to the toxin. In this way the important discovery was made that the immunity was transferable; the properties of an antitoxic blood can be transferred to another individual and an immunity obtained. It was found, however, that in ordinary cases the amount of antitoxin formed, whilst sufficient for the animal originally immunised, was not always adequate for the protection of another animal. The attempt was therefore made to induce the formation of a surplus of antitoxic substances in the blood of the immunised animal, or, in other words, to get it to produce, if possible, more antitoxin than it really wanted for its own requirements. If this were the case, then the

antitoxins became, so to speak, heaped up in the blood, and this excess could then be transferred to and utilised by another animal. It was found that for this purpose the horse is the most useful animal. It can be readily immunised, and large quantities of blood can be removed from it without in any way affecting its health. The first attempts were not successful, the administration of the potent toxin frequently resulting in the death of the animal. Better results were obtained with attenuated toxin, robbed of its full virulence by means of certain agents, *e.g.* heat, or a chemical agent (iodine). Finally, it was found that the immunisation could be effected if the doses of the fresh toxin were carefully graduated and administered at intervals, and this method is now adopted. A small dose produces a reaction, characterised by fever in the animal, but the same dose repeated has no effect, so that one may safely proceed to a larger dose. In this way gradually increasing doses of the toxin can be administered, and a toleration established to doses that in the earlier stages of the immunisation would have proved certainly fatal. This battering of the system with the toxin results in an abnormal activity of the body cells, and a consequently increased output of antitoxic substances which are poured into the blood and accumulate there. When a maximum amount of antitoxin has been in this way obtained, the animal is bled, and the clear serum that separates from the blood is found to contain the larger portion of the antitoxins in solution. They are present in amounts sufficient to protect, not only a large number of animals, but also many human beings from the effects of a diphtheria infection. You will understand how this line of treatment

has been termed serum therapeutics. The antitoxins act not only as preventive agents, they also act as curative agents, so that even when the disease is established in the system they are able to effect a cure, and in this way many thousands of lives have been saved. I must, however, reserve a detailed discussion of antitoxins and serum therapeutics for my next lecture.

LECTURE IV.

Diphtheria—Diphtheria Antitoxin, its Preparation and Use—
Tetanus and Botulism—Ricin and Abrin Immunity—Cholera,
Typhoid and Plague Vaccines.

Serum therapeutics is one of the greatest achievements of Bacteriology, and this lecture will be devoted to a brief consideration of the developments this mode of treatment has up to the present undergone.

We naturally begin with the subject of Diphtheria. I explained to you that the cause of diphtheria is a rod-shaped microbe, the diphtheria bacillus, which grows well on albuminous soils outside the body, and I showed you laboratory cultures of this organism. Its growth in the body is localised, the active seat of its proliferation being the throat. In the throat the bacilli produce a poison, which is readily absorbed into the tissues, and through the action of this poison or toxin the general and fatal symptoms of the disease are brought about. The laboratory cultures of the bacillus contain the toxin, which is probably produced within the bacterial cell, and excreted by it into the surrounding medium. The bacillus is eminently parasitic, and is transferred to others by a patient suffering from the disease, or by objects (such as handkerchiefs, &c.) with which the patient has come in contact. Thus nurses and others in attendance on

diphtheria cases may have the bacilli transferred to their throats, or may by their clothing, &c., transfer the diphtheria organisms to healthy individuals. This points to the importance of the isolation of the patient at home or in a hospital, as well as to the necessity for a stringent disinfection of all contaminated material. The disease is eminently one of childhood, and the disposition to it is marked in the second and third year of life, and begins to decrease in the sixth year. From the eleventh year the disposition to contract the disease is slight, and at the age of fifteen it has almost entirely disappeared. The main features of the former treatment of the disease consisted in the use of antiseptic throat applications, the administration of stimulants and drugs to combat the fever, and the performance of tracheotomy in cases of threatened suffocation. In 1893 Behring's discovery was made of the specific curative serum, which enables one to go to the root of the mischief, and to fight the toxins elaborated by the organism in the body.

The diphtheria serum has a specific action on the diphtheria toxin, and the result it effects is therefore termed a specific immunisation. These specific antitoxic substances are to be found in the blood of individuals who have recovered naturally from the disease. They are, however, to be found in larger quantities in the blood of artificially immunised animals, and we can, by appropriate methods, still further increase their activity and strength, and they are to be found, not only in the blood, but also in the secretions, such as the milk. They circulate through the system and out of it, and disappear in course of time unless replenished. We are in this respect in the

habit of speaking of an *active* immunity and a *passive* immunity. When by the direct action of the toxin on the cells of the tissues the animal is ultimately rendered poison proof—the condition is known as *active* immunity. The main factors in this condition are the body cells, which under the direct action of the toxin respond to the irritation by an increased output of anti-bodies or antitoxins. There has been a direct, active participation of the tissue cells in establishing the immunity—it is thus termed an *active* immunity. When the serum obtained from an animal that has been slowly but surely immunised in this fashion and rendered poison proof is transferred to the system of another animal, its antitoxic action occurs so quickly as apparently to exclude any co-operation of the tissue cells in the result. The anti-toxic bodies are transferred ready formed with the serum, and act without the intervention of the cells of the inoculated animal. The animal owes its protection, its resulting poison-proof condition, not to a direct production of antitoxic substances, but to the utilisation of such bodies indirectly produced for it by another animal.

This condition is known as passive immunity. The horse that yields the antitoxin through injections of toxin is in a state of *active* immunity; the child to which the antitoxin is administered acquires a condition of passive immunity. There is one important difference in these two forms of immunity. The *active* immunity lasts a long time, the passive immunity is of a transient character, and is only available for a momentary emergency in the system.

The artificial immunisation of an animal is brought about by the careful introduction, in the first place, of

minimal doses of the specific poison—doses calculated to produce a disturbance, which is not of a fatal character, in the system. The animal becomes accustomed to these doses, and no effect finally results from their injection. The dose of toxin is gradually increased, and the animal is ultimately in a condition to withstand enormous doses of the poison, thanks to the abnormal and exaggerated output of antitoxic substances. Behring summarised the results obtained as follows: The blood of an animal immunised in this way has a specific destructive action on the toxins of the given disease. These properties are also to be detected in the extra-vascular blood, and in the cell-free serum obtained from it. These properties are of so permanent a nature that they remain active in the blood of another animal, and can effect therapeutic results. Further experiments showed that it was possible to transfer this immunity to man.

The more powerful the toxin, the better and quicker are the immunising results in the horse. In the preparation of a good toxin it is important to have a highly virulent bacillus, or, rather, a good toxin producer, and it is remarkable what differences are met with in this respect. There are certain rare and select strains of the diphtheria bacillus which yield most potent toxins. The Americans do everything on a large scale, and they have succeeded in breeding a diphtheria bacillus which has excited the wonder and envy of all bacteriologists, on account of its marvellous toxin-producing powers, and descendants of this organism are being largely employed in this country for the preparation of diphtheria antitoxin, and the saving of human life. It will, I dare say, be a new

fact to you that a microbe forms one of the beneficent links between the old country and the new. It is important to push the immunity of the horse by means of the toxin to as high a degree as possible, but at the same time not to allow it to reach a point where the animal no longer reacts to injections of the toxin. The slight reactions that occur after injecting the toxin are important, inasmuch as they imply a fresh production of antitoxins in the animal's system. The animal has therefore to be kept in a condition of permanent usefulness—that is, one in which it continues to yield antitoxins. For the first inoculations quite small doses of the *pure* toxin are used. The animal may be regarded as well poison-proof when it can stand sixty to seventy times the amount of the initial dose, and it can then tolerate still larger doses without any injurious effects. A high degree of immunity in the animal is of great value, as the higher its acquired immunity is the less is the dose necessary for the patient. When the horse has reached the proper degree of immunisation, a certain quantity of its blood is removed. The blood is obtained from a vein in the neck of the animal, under antiseptic precautions, and a good many pints may be safely removed at one time. The blood is received in sterile vessels, and kept for some hours in an ice safe; it separates into its solid and fluid constituents. The corpuscular elements are entangled in the clot, and a clear fluid—the serum—rises to the surface. This serum, which contains the bulk of the antitoxic substances, is removed and placed in convenient doses in sterile bottles, which are then sealed up. A small quantity of some antiseptic, such as carbolic acid, is usually added, and the serum is

then ready for use. It is advisable to keep it in a cool and dark place, as light and heat have a tendency to diminish its potency.

Great care is taken to ensure that the horses are absolutely healthy. Each animal before purchase is inspected by a veterinary surgeon, and is also tested with mallein and tuberculin to make certain that it is not suffering from tuberculosis or glanders. The animals, I should like to point out, do *not* suffer by the course of treatment to which they are subjected. After they have been a few months in our hands their condition always improves. One of our first animals came to us as a wretched creature from the shafts of a coster's cart. It is now plump and in good condition, and is ending its days in peace and comfort. When we have to sell an animal we always make a profit on the transaction. Judging by appearances, the animals prefer the occasional prick of a hypodermic syringe to the vicious sting of a whip! What a fine end to the career of a London 'bus horse that, instead of being out on the dreary asphalte in all weathers, it should be utilised as the means of saving the lives of your children! The antitoxin not only acts upon the diphtheria toxin in the body; it also does so in the test-tube. If the proper amount of antitoxin is mixed with the diphtheria toxin in a test-tube, the latter is neutralised and robbed of its poisonous properties; the toxin and antitoxin mutually paralyse each other's action. This mutual action or inter-action occurs proportionally, and can be expressed in figures like an ordinary chemical reaction. A given amount of antitoxin will neutralise a given amount of toxin. It is important to bear in mind that the amount

of the serum itself has nothing to do with its antitoxic properties. A large amount of one serum may have *less* immunising value than a small amount of another serum. The serum of the blood is simply a vehicle for the antitoxic bodies, and may contain them in a dilute or in a concentrated form. The greater the amount of these bodies present the greater is the therapeutic value of the serum. It is therefore of importance that we should be able to estimate the amount of antitoxins present in a serum, in order to gauge its immunising value: in other words, to be able to standardise it. Complicated methods have recently been introduced, to which it will not be necessary to refer on this occasion. The ordinary method of gauging the strength of a serum is based on an arbitrary standard. We, of course, take for this purpose its neutralising action on the toxin. The fatal dose of the toxin is first accurately determined, and then the antitoxin is pitted against *ten* times this fatal dose. We, of course, use the decimal system, and the results are expressed in reference to the fluid unit, the cubic centimetre, or to fractions of the same. A normal serum is one of which $\frac{1}{10}$ cubic centimetre neutralises ten times the fatal dose of the poison, and one cubic centimetre of such a serum is said to contain a normal antitoxin unit. Therefore, a serum of which $\frac{1}{100}$ instead of $\frac{1}{10}$ cubic centimetre balances ten times the fatal dose of the poison has ten times the strength of the other, and one cubic centimetre contains, instead of one, ten normal units. If $\frac{1}{1000}$ cubic centimetre balances ten times the fatal dose, the serum is ten times stronger than the former, and the serum, therefore, contains in one cubic centimetre 100 normal antitoxin units.

I will not attempt to go into this matter further, but sufficient indication has been given of the accurate mathematical control one is able to exercise over the strength of these antitoxic serums for the purpose of gauging their relative value in the treatment of the disease.

At first it was only possible to obtain serums containing 100 to 200 immunising units in the cubic centimetre. Now we can obtain as many as 500 or more units in the cubic centimetre. Such high grade serums are not obtained by concentration of the weaker sorts, but are obtained directly from the animal. In the treatment of diphtheria 1,500 or more units are usually necessary, *i.e.* judged by quality not by quantity. In the case of the older and weaker serums, the injection of large amounts was necessary to ensure an adequate result, and this sometimes produced unpleasant effects, *e.g.* rashes and joint pains, due, not to the antitoxin, but to substances contained in the serum itself. The serums we now possess contain the necessary amount of antitoxins in much smaller bulk, so that temporary discomforts from its use are much less likely to occur. The antitoxins are absolutely harmless to the human body, and act simply as antidotes to the poisons produced in the course of the disease. If, after an animal has been suffering for *twenty-four* hours from a diphtheria infection, the antitoxic serum is administered, one notes a rapid effect upon the local affection. The soft, diffuse œdema becomes a hard circumscribed infiltration, which is thrown off, and the animal recovers its health. This has been effected, not by killing the bacilli, but by producing changes in the tissues which enable them to surmount the degenerative

process at work. Further, the body has been rendered insusceptible to the action of the bacillary poison. If the serum treatment is postponed the local changes may be influenced, but the animal dies. That is to say, whilst the serum has rendered the poison inactive, so much poison had already been taken up by the tissue cells that they succumbed to a slow degeneration. The same is observed in the case of the human being, and you will, therefore, appreciate the importance of an early application of the serum treatment. And further, more serum, or, in other words, stronger antitoxin, is required for the cure than for the prevention of the disease. Dönitz has made some striking experiments which show that the longer the time that elapses between the intoxication and the administration of the serum, the greater is the quantity of serum required for successful treatment. *Eight* minutes after infection with the tetanus poison *six* times the quantity of serum is required to save the animal's life than is necessary if used *immediately after* infection. After one hour *twenty-four* times the original dose of serum is necessary, and so on, until a period is reached when the very strongest serum can no longer save the animal's life. This illustrates amply what I have said as to the importance of early treatment. The antitoxin is given to the patient by means of a syringe, and the amount used for injection depends upon the severity of the case. You will see once more from this that it is not the *quantity* but the *quality* of the serum that guides us in its administration.

For protection a smaller dose is sufficient, *e.g.* 500 units; but the protection lasts only three to four weeks.

Various theories have been advanced as to the action of the antitoxin. Ehrlich considers it to be neutralisation of the toxin in a chemical sense. The toxin and antitoxin unite to form a non-poisonous compound, not only in the body but also in the test tube. Others believe that even this passive immunisation of the body by the ready formed antitoxin is due to a stimulation of the tissue cells—that they are not passive, but directly participate in bringing about the favourable result. I have already indicated that the early administration of the antitoxin is a cardinal point in the treatment of diphtheria. For this an early diagnosis is essential, and this we are able to effect by means of bacteriological methods, which I have already explained to you, and which are now, I am glad to say, largely utilised by medical men. The antitoxin treatment of diphtheria has reduced the mortality from this disease by about two-thirds of that which previously obtained.

Tetanus and botulism are other infections for which antitoxins have been prepared, the principles of preparation and of the treatment of the diseases being the same as those of diphtheria.

This is a convenient place to refer to a poison which, though not formed by bacteria, but by the cells of the animal body, presents close analogies to those we have just been discussing. I refer to snake venom. Snake venom, as you know, has most virulent properties, and, like the tetanus toxin, can exert its action in most minute doses. Fraser found that less than the thousandth part of a gramme of dried cobra venom will kill four guinea-pigs. By beginning with minute, non-

lethal doses of the venom and gradually increasing the quantity Fraser obtained an immunity in an animal whereby it withstood fifty times an otherwise fatal dose of the venom. From rabbits an antitoxic serum was obtained in this manner with which he was able to immunise other rabbits. Fraser terms such blood serum with such properties *Antivenine*. If the serum is injected, even after the symptoms of poisoning have manifested themselves, the animal can be protected and its life saved. The constitution of the poison is unknown, and our tests for it are mainly physiological ones. It diffuses with great rapidity through the system, and acts especially on the nervous centres, producing death by asphyxia. Calmette found that the longer a snake is kept without food the more deadly its poison becomes. Dilute solutions of hypochlorite of lime and of chloride of gold neutralise the snake venom in the test tube. Hypochlorite of lime also neutralises the poison locally in the tissues within twenty minutes after injection. Further, the antivenomous serum neutralises the poison both in the test tube and in the animal body. The snake venom is not a basic or alkaloidal substance; it is rather a substance comparable with the toxins formed by the specific pathogenic organisms—*e.g.* the diphtheria and tetanus toxins. Calmette succeeded in immunising the horse in the same way as has been accomplished in the cases of tetanus and diphtheria. The attenuated and then fully virulent virus was used for this purpose. In the first instance cobra venom, and then a mixture of venoms from other snakes, was injected. He has obtained an antitoxic serum having a strength of 1 in 10,000—*i.e.* rabbits, if given a dose of

venom sufficient to kill in three to four hours, are saved from its fatal effects if a quantity of antitoxic serum corresponding to $\frac{1}{10000}$ part of their weight is injected not later than one to two hours after the injection of the venom. The usual dose of the serum for a man is 10 to 20 cubic centimetres (about 3-6 drachms). About 20,000 people die annually of snake bite in India, and there already exist favourable reports as to the use of this serum.

Ehrlich succeeded in producing immunity in animals to two powerful vegetable poisons, viz. ricin, from the castor-oil bean, and abrin, from the jequirity bean—one gramme of ricin can kill a number of guinea-pigs weighing in the aggregate one and a half million grammes. The animals were given small and gradually increasing doses of these poisons; as a result specific antitoxins developed in their blood, and their serum protected other animals against these poisons. It is interesting to note that the young of an immune mother secure immunity to these poisons through the milk.

We see, therefore, there is a striking similarity in the action of the toxins, whether obtained from a microbe, a plant, or an animal. They act in minute doses, after a shorter or longer incubation period, and they all have the important biological and therapeutic property, in appropriate doses, of producing specific antidotes or antitoxins in the animal body.

Our survey would not be complete without reference to the attempts that have been made to produce a protection against other infectious diseases. These attempts consist mainly in the employment of various vaccines.

For cholera Haffkine has introduced a method of

vaccination. The cholera vaccines are two in number. The first, or weak vaccine, is prepared from attenuated cultures of the cholera bacillus. The second, or strong vaccine, is prepared from cholera cultures of marked virulence. An emulsion is made of the organisms, which is carbolised and preserved in sealed tubes. One cubic centimetre of the weak vaccine is injected under the skin, and the second, or strong vaccine, is injected seven to ten days later. The Haffkine vaccines have been, and are being, extensively used in India as a protection against infection with the cholera organisms, and the results are most hopeful. We are here, of course, dealing not with an antitoxin, but with a vaccine or substance whose action is exerted as a protection against the organisms themselves.

Wright and Semple have also prepared an anti-typhoid vaccine. This vaccine mainly consists in an emulsified culture of the typhoid bacillus, which is sterilised by heating to 60° C. The bacilli are thus killed. One-fourth to one-twentieth of a tube of such a vaccine is administered under the skin. Tenderness occurs at the seat of inoculation, together with more or less marked but transient malaise. It is claimed for this vaccine that it confers an immunity from attacks of typhoid fever, which lasts for a considerable time. Like the cholera vaccine its action is not curative, but preventive.

Haffkine has likewise prepared an anti-plague vaccine. The plague bacillus is grown in a fluid medium, rich in fat, and the culture is freely aerated. After the lapse of a month the culture is heated to 65° C., and the life of the bacillus destroyed. A small quantity of this fluid injected

under the skin constitutes the vaccine. It likewise acts as a protective agent against the bacilli, and encouraging results have been obtained by its use. Yersin attempted to obtain an antitoxic serum for plague, horses being immunised first with dead and then with living cultures of the plague organism. The serum at present obtained is, however, feeble for curative purposes ; perhaps, in course of time, a stronger antitoxic serum will be obtained and a more favourable influence on the disease result from its use.

The streptococci are forms of bacterial life that are responsible for many diseased conditions of the body—such as erysipelas, cellulitis, puerperal fever, and pyæmia ; and also frequently by their presence aggravate the symptoms of other diseases—such as diphtheria and scarlet fever. The attempt has been made to prepare an anti-serum which would successfully combat their effects on the system. The immunisation of horses has been carried out by means of cultures of streptococci of exalted virulence. The living cultures are inoculated into the animal, and after a time the blood of the horse develops certain antitoxic or rather bactericidal properties, as regards the streptococci. The serum was introduced by Marmorek, and it has been used both as a preventive and as a curative agent.

It may be that future research will give us the means of preparing these valuable preventive and curative agents without calling in the aid of the animal body. For the present we know no other satisfactory means of preparing them, and for many years serum therapeutics will occupy the first place in our endeavours to prevent and to cure

disease. We are still on the threshold in this branch of inquiry. Such researches are being actively prosecuted, and I think you will agree with me that we are endeavouring to accomplish a beneficent work and one that has claims on your sympathy and support. Thousands of lives have been saved by the antiseptic treatment in surgery and by the antitoxin treatment in diphtheria.

The methods now adopted for the preparation of antitoxins and vaccines are practically the same as those outlined in the foregoing course of lectures. Though the treatment of diphtheria with antitoxin maintains its superiority over any other, serum or antitoxin treatment of other infective diseases has made little progress during the last ten years, and the hopes which were formerly entertained that antitoxins or anti-serums could be prepared for the cure of such diseases as typhoid fever, plague, cholera, tuberculosis, &c., have not been realised. Owing to this *impasse*, Dr. Macfadyen devoted the last years of his life to attempts to prepare anti-serums with toxins derived from the intra-cellular constituents of bacterial cells, by grinding these in the presence of liquid air (see p. 275).—ED.

THE EFFECTS OF PHYSICAL AGENTS ON
BACTERIAL LIFE

A FRIDAY EVENING DISCOURSE DELIVERED AT
THE ROYAL INSTITUTION, JUNE 8, 1900

Bacteria in the Soil, Air, and Water—Dust Particles in Air—
Effects of Desiccation, Light, Air, Temperature, Electricity,
Agitation, and Pressure on Bacterial Life—Thermophilic
Organisms—Heat Disinfection—Pasteurisation of Milk—Effects
of the Temperature of Liquid Air and of Liquid Hydrogen on
Bacterial Life.

The fact that life did not exist upon the earth at a remote period of time, the possibility of its present existence, as well as the prospect of its ultimate extinction, can be traced to the operation of certain physical conditions. These physical conditions, upon which the maintenance of life as a whole depends, are in their main issues beyond the control of man. We can but study, predict, and it may be utilise their effects for our benefit. Life in its individual manifestations is therefore conditioned by the physical environment in which it is placed. Life rests on a physical basis, and the mainsprings of its energies are derived from a larger world outside itself. If these conditions, physical or chemical, be favourable, the functions of life proceed ; if unfavourable, they cease, and death ultimately ensues. These factors have been studied and their effects utilised to conserve health and to prevent disease. It is our purpose this evening to study some of these purely physical factors, not in their direct bearing on man, but in relation to much lower forms in the scale of life—forms which constitute in number a family far exceeding that of the human species, and of which we may produce at will in a test-tube a population equal to

that of London within a few hours. These lowly forms of life—the bacteria—belong to the vegetable kingdom, and each individual is represented by a simple cell. They are ubiquitous in the soil, air, and water, and are likewise to be met with in intimate association with plants and animals, whose tissues they may invade with injurious or deadly effects. Their study is commonly termed Bacteriology—a term frequently regarded as synonymous with a branch of purely medical investigation. It would be a mistake, however, to suppose that bacteriology is solely concerned with the study of the germs of disease; the dangerous microbes are in a hopeless minority in comparison with the number of those which are continually performing varied and most useful functions in the economy of Nature. The wide importance of bacteria is due to the fact that they ensure the resolution and redistribution of dead and effete organic matter, which, if allowed to accumulate, would speedily render life impossible on the surface of the earth. If Medicine ceased to regard them, their study would still remain of primary importance in relation to many industrial processes in which they play a vital part. It will be seen, therefore, that the biology of bacteria presents many points of interest to scientific workers generally. Their study as factors that intimately concern us really began with Pasteur's researches upon fermentation. The subject of this evening's discourse, the 'Effects of Physical Agents on Bacterial Life,' is important not merely as a purely biological question, though this phase is of considerable interest, but also on account of the facts I have already indicated—viz. that micro-organisms fulfil such an important function in the

processes of Nature, in industrial operations, and in connection with the health of man and animals. It depends largely on the physical conditions to be met with in Nature whether the micro-organisms exercise their functions, or whether they remain inactive or die. Further, the conditions favouring one organism may be fatal to another, or an adaptability may be brought about to conditions unusual for their life. To the technologist the effect of physical agents in this respect is of importance, as a knowledge of their mode of action will guide him to the means to be employed for utilising the micro-organisms to the best advantage in processes of fermentation. The subject is of peculiar interest to those who are engaged in combating disease, as a knowledge of the physical agents that favour or retard bacterial life will furnish indications for the preventive measures to be adopted. To understand the action of physical agents and to call them into full play is to do a work which transcends mere individual effort, and which benefits not one but thousands. It is one of the truest facts that contagious diseases are in large measure preventable, and that any improvement in the physical conditions of man tends to lessen or to stop their propagation. The physical agents which I shall more especially refer to in their effect on bacteria are light, heat, cold, and moisture. With a suitable soil and an adequate temperature the propagation of bacteria proceeds with great rapidity. If the primary conditions of soil and an adequate temperature are not present the organisms will not multiply; they remain quiescent or they die. The surface layers of the soil harbour the vast majority of the bacteria, and constitute

the great storehouse in Nature for these forms of life. They lessen in number in the deeper layers of the soil, and few or none are to be met with at a depth of 8–10 feet. As a matter of fact, the soil is a most efficient bacterial filter, and the majority of the bacteria are retained in its surface layers and are to be met with there. In the surface soil most bacteria find the necessary physical conditions for growth, and may be said to exist there under natural conditions. It is in the surface soil that their main scavenging functions are performed ; in the deeper layers the absence of air and the temperature conditions prove inimical to most forms. There is something, therefore, to be said for the precautions that were taken at the time of the Black Plague, when bodies were ordered to be buried at a depth of 6 feet. Amongst pathogenic bacteria the organisms of lockjaw and of malignant œdema appear to be eminently inhabitants of the soil. As an indication of the richness of the surface soil in bacteria, I may mention that one gram of surface soil may contain from several hundred thousand to as many as several millions of bacteria. The air is poorest in bacteria ; the favouring physical conditions to be met with in the soil are not present in the air. Though bacteria are to be met with in the air, they are not multiplying forms as is the case in the soil. The majority present in air are derived from the soil, and the number lessens when the surface soil is moist, and it increases as the surface soil dries. In a dry season the number of air organisms will tend to increase.

Town air contains more bacteria than country air, whilst they become few and tend to disappear at high levels and on the sea. A shower of rain purifies the

air greatly of bacteria. The organisms being, as I stated, mainly derived from the surface of the ground, their number greatly depends on the physical condition of the soil, and this depends on the weather. Bacteria cannot pass independently to the air; they are forcibly transferred to it with dust from various surfaces. The relative bacterial purity of the atmosphere is mainly, therefore, a question of dust. Even when found floating about in the air the bacteria are to be met with in much greater number in the dust that settles on exposed surfaces, *e.g.* floors, carpets, clothes, and furniture. Through a process of sedimentation the lower layers of the air become richer in dust and bacteria, and any disturbance of dust will increase the number of bacteria in the air—for example, wind, sweeping, &c.

The simple act of quiet breathing does not disseminate disease germs from a patient; it requires an act of speaking, coughing, or sneezing to carry them into the air with minute particles of moisture. A great deal of time and attention has been devoted to the examination of the air for the presence of micro-organisms. When the causal relation of bacteria to different diseases was proved beyond doubt, the air was naturally suspected to be one of the main channels of infection. From the earliest times great weight has been laid upon the danger of infection through air-borne contagia, and with the introduction of antiseptic surgery the endeavour was made to lessen this danger as much as possible by means of the carbolic spray, &c. In the same connection numerous bacteriological examinations of air have been made with the view of arriving at results of hygienic value. The average number

of micro-organisms present in the air is 500 to 1,000 per 1,000 litres. Of this number only 100 to 200 are bacteria, and they are almost entirely harmless forms. The organisms of suppuration have been detected in the air, and the tubercle bacillus in the dust adhering to the walls of rooms. Investigation has not, however, proved air to be one of the important channels of infection in most diseases. The bactericidal action of sunlight, desiccation, and the diluting action of the atmosphere on noxious substances will always greatly lessen the risk of direct aerial infection.

The physical agents that promote the passage of bacteria into the air are inimical to their vitality. Thus, the majority pass into the air not from moist but from dry surfaces, and the preliminary drying is injurious to a large number of bacteria. Their number increases with the amount of dust present, and it follows that if the air is rendered dust-free it is practically deprived of all the organisms it may contain. As regards enclosed spaces, the stilling or laying of dust, and more especially the disinfection of surfaces liable to breed dust or to harbour bacteria, are more important points than air disinfection, and this fact has been recognised in modern surgery. In an investigation, in conjunction with Mr. Lunt, an estimation was arrived at of the ratio existing between the number of dust particles and bacteria in the air. We used Dr. Aitken's dust counter, which not only renders the dust particles visible, but gives a means of counting them in a sample of air. In an open suburb of London we found 20,000 dust particles in 1 c.c. of air; in a yard in the centre of London about 500,000. The dust contamination we found to be about 900 per cent.

greater in the centre of London than in a quiet suburb. In the open air of London there was on an average just one organism to every 38,300,000 dust particles present in the air, and in the air of a room amongst 184,000,000 dust particles only one organism could be detected.¹

These figures illustrate forcibly the poverty of the air in micro-organisms even when very dusty, and likewise the enormous dilution they undergo in the atmosphere. Their continued existence is rendered difficult through the influence of desiccation and sunlight. Desiccation is one of Nature's favourite methods for getting rid of bacteria. Moisture is necessary for their development and their vital processes, and constitutes about 80 per cent. of their cell substance. When moisture is withdrawn most bacterial cells, unless they produce resistant forms of the nature of spores, quickly succumb. The organism of cholera air dried in a thin film dies in three hours. The organisms of diphtheria, typhoid fever, and tuberculosis show more resistance, but die in a few weeks or months.

Dust containing tubercle bacilli may be carried about by air currents, and the bacilli in this way transferred from an affected to a healthy individual. It may, however, be said that drying attenuates and kills most of these forms of life in a comparatively short time. The spores of certain bacteria may, on the other hand, live for many years in a dried condition, *e.g.* the spores of the anthrax bacillus, which are so infective for cattle and also for man (wool-sorters' disease). The spores of the tetanus bacillus, though dried in a thin film on splinters of wood,

¹ Macfadyen and Lunt, *Trans. British Inst. Prev. Med.*, i. p. 142.

have been found virulent after four years. Fortunately few pathogenic bacteria possess spores, and therefore drying by checking and destroying their life is a physical agent that plays an important rôle in the elimination of infectious diseases. This process is aided by the marked bactericidal action of *sunlight*. Sunlight, which has a remarkable fostering influence on higher plant life, does not exercise the same influence on the bacteria. With few exceptions we must grow them in the dark in order to obtain successful cultures, and a sure way of losing our cultures is to leave them exposed to the light of day. Direct sunlight is the most deadly agent, and kills a large number of organisms in the short space of one to two hours. Direct sunlight proves fatal to the typhoid bacillus in half to two hours, to the diphtheria bacillus in half to one hour, and to the tubercle bacillus in from a few minutes to several hours. Even anthrax spores are killed by direct light in three and a half hours. Diffuse light is also injurious, though its action is slower, the typhoid bacillus in one instance dying in about five hours, and the tubercle bacillus in from five to seven days. A similar deleterious action has been demonstrated on other pathogenic organisms and also on the non-parasitic forms. This injurious action of sunlight may be exerted not only on the organisms, but likewise upon the media in which they are present. Some chemical change appears to be produced in the constitution of the nutritive medium, which renders it unsuitable for bacterial growth, *e.g.* the formation of hydrogen peroxide has been suggested. The presence of air hastens the bactericidal action of light. By exposing pigment-producing bacteria to sunlight colourless

varieties can be obtained, and virulent bacteria become so weakened that they will no longer produce infection. The action of sunlight will be most marked on exposed surfaces or on air dust laden with bacteria, weeding out many germs from all places to which it can gain access. The germicidal action of the sun's rays is most marked at the blue end of the spectrum; at the red end there is little or no germicidal action. It is evident that the continuous daily action of the *sun* along with *desiccation* are important physical agents in arresting the further development of the disease germs that are expelled from the body.

It has been shown that sunlight has an important effect in the spontaneous purification of rivers. It is a well-known fact that a river despite contamination at a given point may show little or no evidence of this contamination at a point further down in its course. Buchner added to water 100,000 colon bacilli per cubic centimetre (about sixteen drops), and found that all were dead after one hour's exposure to sunlight. He also found that in a clear lake the bactericidal action of sunlight extends to a depth of about 6 feet. Sunlight must, therefore, be taken into account as an agent in the purification of waters, in addition to sedimentation, oxidation, and the action of algæ. The action of sunlight may be demonstrated in the following way:—Gelatin or agar plates are thickly sown with bacteria, and a portion of the plate is blackened, say, in the form of a cross. The plate is then exposed to sunlight for a short period. The result is that further development of the organisms is arrested in the unprotected but not in the blackened portions of

the plate. The growth assumes the form given to the protected area, *e.g.* a cross or any other selected figure. There are a few exceptions, *e.g.* the *Bacterium photometricum*. If a culture of this organism is illuminated so that the light falls on one point, the organisms collect at the bright point. If a bacillus by chance wanders into the dark area it at once turns back, exhibiting, as it has been termed, 'a movement of alarm.'

Air, or the oxygen it contains, has important and opposite effects on the life of bacteria. In 1861 Pasteur described an organism in connection with the butyric acid fermentation which would only grow in the absence of free oxygen; and since then a number of bacteria, showing a like property, have been isolated and described. They are termed anaërobic bacteria, as their growth is hindered or stopped in the presence of air. The majority of the bacteria, however, are aërobic organisms, inasmuch as their growth is dependent upon a supply of free oxygen. There is likewise an intermediate group of organisms which show an adaptability to either of these conditions, being able to develop with or without free access to oxygen. Pre-eminent types of this group are to be met with in the digestive tract of animals, and the majority of disease-producing bacteria belong to this adaptive class. Whilst the anaërobic organisms grow in the absence of air, yet they require oxygen as much as the aërobic organisms for their life processes, and they obtain it from their food, a favourite form of which is sugar, and sugar therefore constitutes an important element in the laboratory soils used for cultivating anaërobic types of bacteria. When a pigment-producing organism is grown without free oxygen,

its pigment production is almost always stopped. For anaërobic forms nitrogen and hydrogen give the best atmosphere for growth, whilst carbonic acid gas is not so favourable, and may be positively injurious, as in the case of the cholera organism.

As regards *water* in its influence on bacterial life, we may mention a class of organisms, known as the water bacteria, which thrive and multiply in water, and may remain alive for years when planted in this medium. Generally speaking, other species of bacteria, *e.g.* the pathogenic microbes, though they may remain alive for varying lengths of time, do not thrive or multiply in water under ordinary conditions; they remain in a passive not in an active condition. In a pure and naturally cool water the typhoid bacillus may live for about a week; in a water containing organic impurities its length of life may be much longer. Whilst a well-filtered water will not contain more than 100 organisms in 1 c.c., the numbers may rise to 100,000 per c.c. or more in an unfiltered water.

The physical conditions favouring the presence and multiplication of bacteria in water under natural conditions are a low altitude, warmth, abundance of organic matter, and a sluggish or stagnant condition of the water. As regards water-borne infectious diseases, such as typhoid or cholera, their transmission to man by water may be excluded by simple boiling or by an adequate filtration. The freezing of water, whilst stopping the further multiplication of organisms, may conserve the life of disease germs by eliminating the destructive action of commoner competitive forms. Thus, the typhoid bacillus

may remain frozen in ice for some months without injury. The employment of ordinary cold is not, therefore, a protection against dangerous disease germs, whilst the ordinary street ice-creams swarm with a variety of organisms of a more or less unsavoury description.

As regards *electricity*, there is little or no evidence of its direct action on bacterial life; the effects produced appear to be of an indirect character due to the development of heat or to the products of electrolysis. Thus, in a solution containing salt there is a development of acid and alkali, and free chlorine and hypochlorites may be formed. Hydrogen peroxide and ozone may likewise develop in the fluid.

Ozone is a powerful disinfectant, and its introduction into polluted water has a most marked purifying effect. The positive effects of the electric current may, therefore, be traced to the action of the chemical products and of heat. I am not aware that any direct action of the *X*-rays on bacteria has up to the present been definitely proved.

Mechanical agitation, if slight, may favour, and if excessive may hinder bacterial development. Violent shaking or concussion may not necessarily prove fatal so long as no mechanical lesion of the bacteria is brought about. If, however, physical agents likely to produce triturating effects are introduced, a disintegration and death of the cells follow. Thus, Rowland, by a very rapid shaking of tubercle bacilli in a steel tube with quartz sand and hard steel balls produced their complete disintegration in ten minutes.

Bacteria appear to be very resistant to the action of *pressure*. At 300 to 450 atmospheres putrefaction still

takes place, and at 600 atmospheres the virulence of the anthrax bacillus remains unimpaired.

Of the physical agents that affect bacterial life *temperature* is the most important. Temperature profoundly influences the activity of bacteria. It may favour or hinder their growth, or it may put an end to their life. If we regard temperature in the first instance as a favouring agent, very striking differences are to be noted. A general rule cannot be laid down for the bacteria as a class, as each species has its own conditions of temperature under which its vital processes are most actively manifested. The bacteria show a most remarkable range of temperature under which their growth is possible, extending from zero to 70° C. If we begin at the bottom of the scale we find organisms are to be met with in water and in soil that are capable of growth and development at zero Centigrade. Amongst these are certain species of phosphorescent bacteria which continue to emit light even at this low temperature. At the Jenner Institute we have met with organisms growing and developing at 1° to 4° C. The vast majority of interest to us find, however, the best conditions for their growth from 15° up to 37° C. Each species has a minimum, an optimum, and a maximum temperature at which it will develop. It is important in studying any given species that the optimum temperature for development be ascertained, and that this temperature be maintained. In this respect we can distinguish three broad groups. The first group includes those for which the optimum temperature is from 15° to 20° C. The second group includes the parasitic forms, viz. those which grow in the living body and for which the optimum

temperature is at blood heat, viz. 37° C. We have a third group for which the optimum temperature lies as high as 50° to 55° C. This latter group has been termed 'thermophilic,' on account of its preference for growing at such abnormally high temperatures—temperatures which are fatal to most other forms of life. A few words may be devoted to thermophilic bacteria, as they are of great biological interest. Some years ago Miguel isolated a bacillus which grew at 70° C., to which he applied the name *B. thermophilus*, and the existence of such forms of life was subsequently confirmed by Globig. They have been the subject of personal investigation in conjunction with Dr. Blaxall.¹ We found that, so far from the *B. thermophilus* being an isolated example of bacterial life at an abnormally high temperature, there existed in Nature an extensive group of such organisms to which the term 'thermophilic bacteria' was applicable. Their growth and development occurred best at temperatures at which ordinary protoplasm becomes inert or dies. I must confess that we followed the life history of these strange organisms with considerable fascination and interest. The best growths were always obtained at 55° to 65° C. Their wide distribution was of a striking nature. They were found by us in river water and mud, in sewage, and also in a sample of sea water. They were present in the digestive tract of man and animals and in the surface and deep layers of the soil, as well as in straw and in all samples of ensilage examined. Their rapid growth at high temperatures was remarkable, the whole surface of the culture medium being frequently overrun in from fifteen to

¹ Macfadyen and Blaxall, *Journ. of Pathol. and Bacteriol.*, 1894.

seventeen hours. The organisms examined by us (fourteen forms in all) belonged to the group of the bacilli. Some were motile, some curdled milk, and some liquefied gelatin in virtue of a proteolytic enzyme. The majority possessed reducing powers upon nitrates, and decomposed proteid matter. In some instances cane sugar was inverted and starch was diastased. These facts well illustrate the full vitality of the organisms at these high temperatures. Whilst all the organisms isolated grew best at 55° to 65° C., a good growth in a few cases occurred at 72° C., and evidence of growth was obtained even at 74° C. They exhibited a remarkable and unique range of temperature, extending through 30° of the Centigrade scale. However, they are able to exist, and there is little doubt with regard to the favouring influence of the physical factor of a high temperature on these ubiquitous organisms. Ensilage kept at 60° C. in a hermetically sealed box was examined at the end of two months: whilst the ordinary bacteria were found to be killed, the ensilage was, on the other hand, swarming with active thermophilic bacteria even after this prolonged cooking at 60° C. As a concluding instance of the activity of these organisms, we may cite their action upon cellulose. Cellulose is a substance that is exceedingly difficult to decompose, and is therefore used in the laboratory for filtering purposes in the form of Swedish filter-paper on account of its resistance to the action of solvents. We allowed these organisms to act on cellulose at 60° C. The result was that in ten to fourteen days a complete disintegration of the cellulose had taken place, probably into carbonic acid and marsh gas. In how far can such organisms find in Nature the temperatures

that are so well suited to their growth? In a silo we examined the temperature reached 60° C., and in pits and heaps of manure 60° to 70° C. is often attained in the course of their spontaneous fermentation; and in other fermentations associated with bacteria high temperatures have been noted, *e.g.* from 40° up to 70° C. In the heating of hay-ricks the temperature rises, through bacterial agency, to 50° and 60° C. One is sometimes apt to forget the amount of heat that is being continually developed at all seasons of the year by the processes of Nature, temperatures which will favour the development of thermophilic organisms. The exact conditions that may favour their growth, even if it be slow at subthermophilic temperatures, are not yet known; they may possibly be of a chemical nature.

Organisms may be gradually *acclimatised* to temperatures that prove unsuited to them under ordinary conditions. Thus the anthrax bacillus, with an optimum temperature for its development of 37° C., may be made to grow at 12° C. and at 42° C. Such anthrax bacilli proved pathogenic for the frog, with a temperature of 12° C., and for the pigeon with a temperature of 42° C.

Let us in a very few words consider the inimical action of temperature on bacterial life. An organism placed below its minimum temperature ceases to develop, and if grown above its optimum temperature it becomes attenuated as regards its virulence, &c., and may eventually die. The boiling point is fatal for non-sporing organisms in a few minutes. The exact thermal death point varies according to the optimum and maximum temperatures for the growth of the organism in question. Thus for water

bacteria with a low optimum temperature blood heat may be fatal ; for pathogenic bacteria developing best at blood heat a thermophilic temperature may be fatal (60° C.) ; and for thermophilic bacilli any temperature above 75° C. These remarks apply to the bacteria during their multiplying and vegetative phase of life ; in their resting or spore stage the organisms are much more resistant to heat. Thus the anthrax organism in its bacillary phase is killed in one minute at 70° C. ; in its spore stage it resists this temperature for hours, and is only killed after some minutes by boiling. In the soil there are spores of bacteria which require boiling for sixteen hours to ensure their death, and even at 105° to 110° C. are only killed in from two to four hours. These are important points to be remembered in sterilisation or disinfection experiments—viz. whether an organism does or does not produce these resistant spores. The tenacious theory of spontaneous generation was in a measure simply due to faulty experimental methods, which overlooked the fact that a simple boiling does not kill all forms of bacterial life. Most non-sporing forms are killed at 60° C. in a few minutes, but in an air-dry condition a longer time is necessary to coagulate and kill their protoplasm. Dry heat requires a longer time to act than moist heat, and has little penetrating power compared with steam ; it requires 140° C. for three hours to kill anthrax spores. Dry heat cannot, therefore, be used for ordinary disinfection on account of its destructive action on the articles to be disinfected. Moist heat in the form of steam is the most effectual disinfectant, killing anthrax spores at boiling point in a few minutes, whilst a still quicker action is obtained if saturated steam under

pressure be used. No spore, however resistant, remains alive after one minute's exposure to steam at 140° C.

The varying thermal death point of organisms and the problems of sterilisation cannot be better illustrated than in the case of milk, which is an admirable soil for the growth of a large number of bacteria. The most obvious example of this is the souring and curdling of milk that occurs after it has been standing for some time. This change is mainly due to the lactic acid bacteria, which ferment the milk sugar with the production of acidity.

Another class of bacteria may curdle the milk without souring it in virtue of a rennet-like ferment, whilst a third class, mainly spore-bearing forms, precipitates and dissolves the casein of the milk, along with the development of butyric acid. The process whereby milk is submitted to a heat of 65° to 70° C. for twenty minutes is known as Pasteurisation, and the milk so treated is familiar to us all as Pasteurised milk. Whilst the Pasteurising process weeds out the lactic acid bacteria from the milk, a temperature of 100° C. for one hour is necessary to destroy the butyric acid organisms. And even when this has been accomplished, there still remain in the milk the spores of organisms, belonging to the group of the hay bacilli, which are only killed at a temperature of 100° C. in from three to six hours. It will, therefore, be seen that Pasteurisation produces a partial, not a complete, sterilisation of the milk as regards its usual bacterial inhabitants. The sterilisation to be absolute would require six hours at boiling point, or a shorter period with steam under pressure. But for all ordinary practical purposes Pasteurisation is an adequate

procedure. All practical hygienic requirements are likewise adequately met by Pasteurisation, if it is properly carried out and the milk is subsequently cooled. Milk may carry the infection of diphtheria, cholera, typhoid and scarlet fevers as well as the tubercle bacillus from a diseased animal to the human subject. For the purpose of rendering the milk innocuous freezing and the addition of preservatives are inadequate methods of procedure. The one efficient and trustworthy agent we possess is heat. Heat and cold are the agents to be jointly employed in the process—viz. a temperature sufficiently high to be fatal to organisms producing a rapid decomposition of milk, as well as to those which produce disease in man. This to be followed by a rapid cooling to preserve the fresh flavour and to prevent an increase of the bacteria that still remain alive. The Pasteurising process, if properly applied, fulfils these requirements. The process may be carried out by passing the milk in a thin stream over a heated metal surface into a cooler, or it may be heated in bulk by steam to the desired temperature and then cooled. Such machines will destroy about 70 per cent. of the ordinary bacteria, but pathogenic microbes may not be killed with certainty.

In conjunction with Dr. Hewlett,¹ I had occasion to investigate in how far the best Pasteurising results might be obtained. We found that 60° to 68° C. applied for twenty minutes weeded out about 90 per cent. of the organisms present in the milk, leaving a 10 per cent. residue of resistant forms or their spores. It was found advisable to fix the Pasteurising temperature

¹ Macfadyen and Hewlett, *Trans. British Inst. Prev. Medicine*, i. p. 82.

at 68° C. in order to make certain of killing any pathogenic organisms that may happen to be present. In our experiments we passed milk in a thin stream through a coil of metal piping, which was heated on its outer surface by water. By regulating the length of the coil, or the size of the tubing, or the rate of flow of the milk almost any desired temperature could be obtained. The temperature we ultimately fixed at 70° C. The cooling was carried out in similar coils of tubing placed in iced water. The thin stream of milk was quickly heated and quickly cooled as it passed through the heated and cooled tubing, and whilst it retained its natural flavour the apparatus accomplished at 70° C. in thirty seconds a complete Pasteurisation instead of in twenty minutes—*i.e.* about 90 per cent. of the bacteria were killed, whilst the diphtheria, typhoid, tubercle and pus organisms were destroyed in the same remarkably short period of time, viz. thirty seconds. This will serve to illustrate how the physical agent heat may be employed, as well as the sensitiveness of bacteria to heat when this is adequate. Here we have a temperature at which thermophilic organisms will grow, proving entirely and quickly destructive to many other forms of bacterial life.

Bacteria are much more sensitive to high than to low temperatures, and it is possible to proceed much further downwards than upwards in the scale of temperature without impairing their vitality. Some will even multiply at zero, whilst others will remain alive when frozen under ordinary conditions.

I will conclude this discourse by briefly referring to experiments made with most remarkable results upon

the influence of low temperatures on bacterial life.¹ The experiments were conducted at the suggestion of Sir James Crichton Browne and Professor Dewar. The necessary facilities were most kindly given at the Royal Institution, and the experiments were conducted under the personal supervision of Professor Dewar. The action of liquid air on bacteria was first tested. A typical series of bacteria was employed for the purpose, possessing varying degrees of resistance to external agents. The bacteria were first simultaneously exposed to the temperature of liquid air for twenty hours (about -190° C.). In no instance could any impairment of the vitality of the organisms be detected as regards their growth or functional activities. This was strikingly illustrated in the case of the phosphorescent organisms tested. The cells emit light which is apparently produced by a chemical process of intracellular oxidation, and the phenomenon ceases with the cessation of their activity. These organisms, therefore, furnished a very happy test of the influence of low temperatures on vital phenomena, and when cooled down in liquid air they became non-luminous, but on re-thawing the luminosity returned with unimpaired vigour as the cells renewed their activity. The sudden cessation and rapid renewal of the luminous properties of the cells, despite the extreme changes of temperature, were remarkable and striking. In further experiments the organisms were subjected to the temperature of liquid air for seven days. The results were again *nil*, for on re-thawing the organisms renewed their life processes with unimpaired

¹ Macfadyen, *Proc. Roy. Soc. Lond.*, vol. lxvi., 1900, pp. 180 and 489, *ib.* vol. lxxi., 1902, p. 76.

vigour. We had not yet succeeded in reaching the limits of vitality. Professor Dewar kindly afforded the opportunity of submitting the organisms to the temperature of liquid hydrogen, about -250° C. The same series of organisms was employed, and again the result was *nil*.¹ This temperature is only 23° above that of the absolute zero, a temperature at which in our present theoretical conceptions molecular movement ceases, and the entire range of chemical and physical activities with which we are acquainted either ceases, or may assume an entirely new phase. This temperature again is far below that at which any chemical reaction is known to take place. The fact, then, that life can continue to exist under such conditions affords new ground for reflection as to whether, after all, life is dependent for its continuance on chemical reactions. We, as biologists, therefore follow with the keenest interest Professor Dewar's heroic attempts to reach the absolute zero of temperature. Meanwhile his success has already led us to reconsider many of the main issues of the problem. And by having afforded us a new realm in which to experiment, Professor Dewar has placed in our hands an agent of investigation from the effective use of which we who are working at the subject at least hope to gain a little further insight into the great mystery of life itself.

¹ Macfadyen and Rowland, *Proc. Roy. Soc. Lond.*, vol. lxvi. p. 488.

GLOSSARY

Actinia.—The sea anemone, &c.

Algæ.—Seaweeds and fresh-water weeds. Plants without distinction between root and stem.

Amœba.—A minute, naked-celled protozoon with pseudopodia, often found in stagnant water. The form of the cell is continually altering (hence the term ‘amœboid movement’).

Arcella.—A multi-nucleated protozoon in which the pseudopodia are confined to one region of the body, the rest of the body being enclosed in a shell.

Characeæ.—An order of lowly water plants with a distinct axis branching in a whorled manner.

Cœlenterata.—A group of the lower Metazoa containing those animals commonly known as polyps, *e.g.* jelly fish, sea anemones, and corals.

Foraminifera.—Protozoa having calcareous shells perforated by numerous pores.

Heliozoa.—A group of Protozoa, naked or with a siliceous skeleton, and having numerous stiffish filiform pseudopodia, *e.g.* *Actinophrys*, the ‘sun-animalcule.’

Hydra.—The fresh-water polyp.

Infusoria.—A group of the Protozoa in which the cell is provided with numerous short bristles or cilia. The common ‘animalcules’ of stagnant water are mostly Infusoria.

Leguminosæ.—An order of plants to which the peas, beans, and vetches belong.

Leucocytes.—The white or colourless corpuscles of the blood.

Metazoa.—See ‘Protozoa.’

- Myxomycetes*.—The 'slime fungi.' Plant organisms the body of which consists of an amœboid mass of protoplasm with many nuclei. Found on decaying vegetable matter.
- Paramœcium*.—The 'slipper animalcule,' so called from its shape. A ciliated protozoon common in ditches.
- Protococcus*.—A minute, unicellular, chlorophyll-containing plant common in ditches and rain-water.
- Protozoa*.—The animal kingdom is divided into two great groups, the Protozoa and Metazoa. The former are unicellular, the latter multicellular, organisms.
- Pseudopodia*.—The irregular extrusions or processes of protoplasm occurring on naked-celled Protozoa and white blood corpuscles.
- Radiolaria*.—Protozoa with stiffish, filiform, anastomosing pseudopodia radiating from all sides of the globular body, which usually has a delicate and often elaborate siliceous skeleton.
- Rhizopoda*.—Minute Protozoa possessing a skeleton formed of silica.
- Siphonophora*.—A group of the Cœlenterata or polyps, forming free-swimming colonies.
- Spirogyra*.—An alga in which the chlorophyll bodies are arranged spirally in the cells.
- Symbiosis*.—The living together of two or more organisms for their mutual benefit (*e.g.* lichens, fungus + alga), or for bringing about certain chemical changes (*e.g.* the ginger-beer plant, yeast + bacterium).
- Thallus*.—A combination of similar cells to form a leaf-like expansion, without differentiation into leaf, stem, and root, found in the lower plants, such as the algæ, fungi, and lichens.
- Tradescantia*.—An alga which shows marked circulation of the protoplasm of its cells.
- Vampyrella*.—A protozoon with filamentous pseudopodia, parasitic on diatoms.
- Volvox*.—Microscopic ciliated organisms containing chlorophyll, occurring in fresh water. By some regarded as Protozoa, by others as Algæ (see Plate, p. 43).
- Vorticella*.—A bell-shaped, ciliated protozoon, with retractile stalk, common in ditches.

PUBLISHED PAPERS

The following are some of the principal papers published by the late Dr. Allan Macfadyen :—

The Behaviour of Bacteria in the Digestive Tract. *Journ. of Anat. and Physiol.*, vol. xxi., 1886–87, p. 227.

[This is believed to be the first paper published by Dr. Macfadyen. It embodies the substance of a Thesis presented to the Medical Faculty of the University of Edinburgh for graduation as M.D., and for which a gold medal was awarded to the author, August 2, 1886.—ED.]

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